University of Alberta

Biosynthetic Studies on the Polyketide Lovastatin: Enzyme-Catalyzed Diels-Alder Reactions

by

Karine Auclair ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry

Edmonton, Alberta, CANADA

Fall 1999

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-46802-X

Canada

University of Alberta

Library Release Form

Name of author: Karine Auclair

Title of Thesis: Biosynthetic Studies on the Polyketide Lovastatin: Enzyme-Catalyzed Diels-Alder Reactions

Degree: Doctor of Philosophy

Year this degree granted: 1999

Permission is hereby granted to the University of Alberta to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication rights and all other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Karine Auclair

Permanent address:
3408 St-Dominique
Jonquière, Québec
Canada, G7X 7V6

Dated: Sept 15/99

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies and Research for acceptance, a thesis entitled Biosynthetic Studies on the Polyketide Lovastatin: Enzyme-Catalyzed Diels-Alder Reactions submitted by Karine Auclair in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

D. L. J. Clive

Dr. Derrick L. J. Clive

Dr. Dennis G. Hall

C R Hutchinson

Dr. C. Richard Hutchinson (External)

Dr. Susan Jensen

George Kotovych

Dr. George Kotovych

Dr. John C. Vederas (Supervisor)

Dated: Sept 10, 1999

Je tiens à dédier cette thèse à ma belle petite maman Lise.

Par ta patience, ta droiture, ta curiosité, et ta vertu, tu m'as offert un superbe exemple.

Merci maman pour ton écoute impartiale, tes conseils sages, ainsi que pour les belles valeurs et leçons de vie que tu m'as transmises.

À travers les nombreuses étapes de cette recherche,
ton support m'a donné les ailes pour persévérer dans la noirceur.

Cet ouvrage t'est dédié avec amour.

Que mes souhaits de bonheur, de santé, et de croissance t'habitent tout au long du chemin.

ABSTRACT

The biosynthesis of lovastatin (**1**) by *Aspergillus terreus* was examined using *Penicillium aurantiogriseum* which produces compactin (**5**), a 6-desmethyl analogue of **1**, and mutated fungal strains prepared by Professor Hutchinson (University of Wisconsin). [2,3-$^{13}C_2$]-3-Acetoacetic acid , *N*-acetylcysteamine thioester (**28a**) and [2,3-$^{13}C_2$]-3-hydroxybutyric acid, *N*-acetylcysteamine thioester (**29a**) are not incorporated into **5**.

Aspergillus nidulans lovB, a strain in which the lovastatin nonaketide synthase (LNKS, *lovB*) gene from *A. terreus* has been overexpressed, produces 4-hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (**41**) and 4-hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (**42**). ^{13}C-Labeled sodium acetate is incorporated into **41** and **42** at a rate of 70% rate.

A. nidulans lovB+lovC, a strain in which both *lovB* and *lovC* genes have been overexpressed, produces 4a,5-dihydromonacolin L (**11**) both *in vivo* and *in vitro*. ^{13}C-Labeled sodium acetate is incorporated into **11** with an efficiency of 20%, but neither **29a** nor [2,11-$^{13}C_2$]-(2*E*,8*E*,10*E*)-(*R*)-6-methyldodecatri-2,8,10-enoic acid, *N*-acetylcysteamine thioester (**16a**) are transformed into **11** using intact cells or cell free systems.

A. terreus lovC, a strain in which the *lovC* gene has been inactivated, produces **41** and **42**, but not **1**, nor **11**. This strain allows successful incorporations of monacolin J into **1**, 6-desmethylmonacolin J into **5**, and **11** into **1**, but not of ML-236C into **5**.

LNKS was purified and tested for Diels-Alderase activity. LNKS catalyzes the Diels-Alder reaction of **16** with the correct stereochemistry required for the biosynthesis of **1**. LNKS does not catalyze the Diels-Alder cyclizations of [3-carbomethoxy-(*E*)-2-propen-1-oxy]-(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (**59**) or *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (**60**).

Model studies on potential enzyme-catalyzed Diels-Alder reactions were undertaken. Compounds **59** and **60** remain unchanged when reacted with alcohol dehydrogenase, subtilisin Carlsberg, or carbonic anhydrase. [4-Hydroxy-(*E*)-2-buten-1-oxy]-(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane **78** is not cyclized by alcohol dehydrogenase. 2-(4-Penten-1-yl)phenol (**82**), 2-(5-hexen-1-yl)phenol (**83**), 4-(pent-4-enyl)phenol (**92**), 4-(5-hexen-1-yl)phenol(**93**), and 4-(6-hepten-1-yl)phenol (**94**) were synthesized and reacted with tyrosinase. Compounds **82** and **83** are not transformed by this enzyme, whereas **92**, **93**, and **94** are oxidized to *ortho*-quinones which polymerized before an intramolecular Diels-Alder reaction can occur. The reaction of **92** with tyrosinase in presence of ethyl vinyl ether affords the intermolecular Diels-Alder adduct *exo*-7-ethoxy-6-(5-hexen-1-yl)bicyclo[2.2.2]-2,3-dioxo-octa-5-ene. Ethyl 7-(4-methoxyphenyl)-2*E*-hexenoate, 6-ethoxy-1-(4-methoxyphenyl)-5-hexene, 6-(4-methoxyphenyl)-1-hexyne, and 1-(3,4-dimethoxyphenyl)-5*E*,6-octadiene were prepared, but deprotection of the methyl ethers to give the corresponding phenols proved difficult.

Acknowledgements

I thank my supervisor, Professor John C. Vederas, for his enthusiasm, advice, and support through this life experience. I would like to express my gratitude to Dr. Andrew Sutherland and Mr. John L. Sorensen for their help and proof-reading of this manuscript. I am grateful to the past and present research group members for their help at various points. I would also like to thank Professor Hutchinson and his research group members at the University of Wisconsin for sharing their knowledge and expertise in molecular biology. The staff of spectral and analytical services are also thanked for their assistance in characterizing compounds.

The Natural Sciences and Engineering Research Council of Canada, the Alberta Heritage Foundation for Medical Research, and the University of Alberta are gratefully acknowledged for financial support.

Finally, I would like to thank all my friends and family members for their moral support and encouragement.

Table of Contents

	Page
INTRODUCTION	1
1. Biosynthesis of Polyketides: from Chemistry to Molecular Biology	1
2. Lovastatin and Related Cholesterol-Lowering Drugs	12
3. Biosynthesis of Lovastatin	18
4. Recent Advances in Chemical and Enzymatic Diels-Alder Reactions	22
RESULTS AND DISCUSSION	31
1. Molecular Biology of Lovastatin Biosynthesis	31
1.1 Sequencing of the lovastatin gene cluster by Professor Hutchinson at the University of Wisconsin	31
1.2 Genetically engineered fungal strains prepared by Professor Hutchinson at the University of Wisconsin	33
2. Incorporation Experiments with Intact Cells for the Elucidation of Lovastatin and Compactin Biosynthesis	35
2.1 Incorporation studies into compactin using *Penicilium aurantiogriseum*	35
2.2 Studies with *A. terreus lovE* and *A. terreus* XS-1P strains	42
2.3 Production of novel metabolites by the *A. nidulans lovB* strain	44
2.4 Production of a new metabolite by the *A. terreus lovC* mutant	51
2.5 The *A. terreus lovA* mutant does not produce 4a,5-dihydromonacolin L	52
2.6 Inhibition of *A. terreus* XS-1P with ancymidol	52
2.7 Production of 4a,5-dihydromonacolin L by the *A. nidulan lovB+lovC* strain	54
2.8 Incorporation studies of a diketide into 4a,5-dihydromonacolin L	

using the *A. nidulans lovB+lovC* strain..... 56

2.9 Significance..... 57

3. Studies on Post-PKS Transformations Leading to Lovastatin..... 58

3.1 Production of monacolin J by the *A. terreus lovD* and *lovF* mutants..... 58

3.2 Investigation of the production of the side chain diketides in the *A. terreus lovC* mutant..... 59

3.3 Investigation of incorporation of monacolin J into lovastatin using the *A. terreus lovC* mutant..... 60

3.4 Investigation of the incorporation of desmethylmonacolin J into compactin using the *A. terreus lovC* mutant..... 61

3.5 Incorporation of 4a,5-dihydromonacolin L into lovastatin using the *A. terreus lovC* mutant..... 62

3.6 Attempted incorporation of ML-236C into compactin using the *A. terreus lovC* mutant..... 62

3.7 Significance..... 62

4. Cell Free Experiments for the Study of Lovastatin Nonaketide Assembly by PKS Enzymes..... 64

4.1 Cell free extracts of *A. terreus lovE* and *A. terreus* XS-1P..... 64

4.2 Cell free extracts of *A. nidulans*..... 65

4.3 Cell free production of 4a,5-dihydromonacolin L..... 66

4.4 Attempted incorporation of a diketide into 4a,5-dihydromonacolin L using cell free extracts of the *A. nidulans lovB+lovC* strain..... 67

4.5 Attempted incorporation of a triene into 4a,5-dihydromonacolin L using cell free extracts of the *A. nidulans lovB+lovC* strain..... 68

4.6 Studies on the use of propionyl coenzyme A and butyryl coenzyme A as starter units by cell free extracts of the *A. nidulans*

lovB+lovC strain..... 69
4.7 Significance..... 71
5. Enzyme-Catalyzed Diels-Alder Reactions..... 71
5.1 Lovastatin nonaketide synthase..... 73
5.2 LNKS enzyme purification..... 73
5.3 LNKS-catalyzed Diels-Alder reaction..... 74
5.4 Synthesis of alternative trienes for exploration of the substrate specificity of LNKS..... 77
5.5 Investigation of subtilisin Carlsberg as a possible Diels-Alderase.. 82
5.6 Investigation of carbonic anhydrase as a possible Diels-Alderase .. 84
5.7 Investigation of alcohol dehydrogenase from horse liver or from Baker's yeast as a possible Diels-Alderase..... 86
5.8 Investigation of tyrosinase as a possible Diels-Alderase..... 88
5.9 Significance..... 105
EXPERIMENTAL..... 107
1. General..... 107
2. Experimental Data for Compounds..... 110
3. Fermentation and Incorporation Methods..... 192
4. Cell Free Extracts and LNKS Purification..... 209
5. Enzymatic Tests for Compounds..... 216
REFERENCES..... 230

List of tables

Table		Page
1.	Proposed proteins encoded by the genes present in the biosynthesis cluster of lovastatin.....	32
2.	Genetically engineered fungal strains used in this work.....	34

List of Figures

Figure		Page
1.	Lovastatin	1
2.	Examples of polyketides	2
3.	Biosynthesis of polyketides	3
4.	Chain termination in the biosynthesis of 6-methylsalicylic acid (**2**)	4
5.	The biosynthesis of the polyketide portion of erythromycin, 6-deoxyerythronolide B, by deoxyerythronolide synthase	5
6.	The fungal polyketides dehydrocurvularin (**4**), oudenone and aspyrone	7
7.	Erythomycin A, tetracenomycin C and daunorubicin	8
8.	The non-ribosomal peptide cyclosporin A contains the polyketide portion BmT	8
9.	DnrD cyclization of aklanonic acid methyl ester (AAME) to aklaviketone (AK) during the biosynthesis of doxorubicin	10
10.	Intermodular communication in polyketise synthases	11
11.	Structures of lovastatin (**1**) and compactin (**5**)	13
12.	HMG-CoA reductase inhibitors	14
13.	The biosynthesis of cholesterol and the mechanism of HMG-CoA reductase	16
14.	Inhibitors of squalene synthase (SS)	18
15.	Origin of the atoms in lovastatin (**1**)	19
16.	Effects involved in the acceleration of the Diels-Alder reaction by water	25
17.	Lovastatin gene cluster	31
18.	Compactin production curve and growth curve of *P. aurantiogriseum*	36
19.	Dehydrocurvularin (**4**) and β-oxidation inhibitors **26** and **27**	38
20.	Diketides **28**, **28a**, **29**, and **29a**	39

21. Tetraketides **37** and **38** for incorporation studies with dehydrocurvularin (**4**)..... 43
22. The yellow pigments **41** and **42**..... 46
23. Results of selected 2D NMR experiments on compounds **41** and **42**.... 48
24. Cytochrome P_{450} dependant enzyme inhibitors..... 53
25. Results of selected 2D NMR experiments on compounds **11** and **11a**.. 55
26. X-ray crystal structure of compound **11**..... 55
27. Monacolin J (**14**)..... 58
28. Compounds **11**, **12**, **13**, **45**, and **46**..... 59
29. Tiglic acid (**50**) and crotonic acid (**51**)..... 60
30. Lovastatin (**1**) and monacolin J (**14**)..... 61
31. ML-236C (**24**) is an intermediate in the pathway towards compactin (**4**) 63
32. Examples of polyketides whose biosyntheses have been studied using cell free extracts..... 67
33. The diketide **29a** and 4a,5-dihydromonacolin L (**11**)..... 68
34. Hexaketide **16** and **16a**..... 69
35. Prosolanapyrone II (**23**) and solanapyrone A (**16**)..... 72
36. Elution curve and SDS-PAGE of the pooled protein samples collected during size exclusion chromatography in the purification of LNKS..... 74
37. The *N*-acetylcysteamine thioesters **70** and **71**..... 81
38. Target compounds **128**, **129**, **130**, and **131**..... 101

List of Schemes

Scheme		Page
1.	Post-PKS transformations leading to lovastatin (**1**).....	20
2.	Proposed pathway for the biosynthesis of 4a,5-dihydromonacolin L (**11**)	21
3.	The four possible diastereomeric products resulting from the Diels-Alder reaction of the postulated intermediate triene.....	22
4.	The biosynthesis of solanapyrones involves an enzyme-catalyzed Diels-Alder reaction.....	28
5.	Reactions catalyzed by tyrosinase.....	29
6.	Enzyme-initiated hydroxylation-oxidation-Diels-Alder reaction cascade	30
7.	Mechanism of β-oxidation.....	37
8.	Synthesis of diketide **29a**.....	40
9.	Synthesis of [1,2-$^{13}C_2$]-acetyl CoA (**39a**).....	44
10.	Hydrogenation of compounds **41** and **42**.....	49
11.	Proposed biosynthesis of compounds **41** and **42**.....	50
12.	Compactin (**5**) and 6-desmethylmonacolin J (**52**).....	61
13.	Precursor-directed biosynthesis of 4a,5-dihydromonacolin L derivatives.....	70
14.	Demonstration of LNKS-catalyzed Diels-Alder reaction.....	76
15.	Synthesis of trienes **59** and **60**.....	78
16.	Thermal Diels-Alder reaction of compounds **59** and **60**.....	79
17.	Suggested alternative assay for Diels-Alder adducts of **59** and **60**.....	80
18.	Tentative preparation of compounds **70** and **71**.....	82
19.	Activation of carbonyl functionalities by serine proteases.....	84
20.	Reaction catalyzed by carbonic anhydrase.....	84
21.	Synthesis of alcohol **78** and reactivity of aldehyde **79**.....	87

22. Attempted synthesis of alcohol **81** 88
23. Enzyme-initiated hydroxylation-oxidation-Diels-Alder reaction cascade 90
24. Synthesis of *ortho*-phenol **82** 91
25. Synthesis of *ortho*-phenol **83** 92
26. Proposed mechanism for the formation of compounds **83** and **88** 93
27. Target compounds **92**, **93**, and **94**, their oxidation products, and possible Diels-Alder adducts 94
28. Approaches to the synthesis of compound **92** 95
29. Attempted Fries rearrangement of **103** 96
30. Alkylation of phenol with succinic anhydride 96
31. Attempted formation of **110** via organometallic reactions 97
32. Synthesis of target compounds **92**, **93**, and **94** 98
33. Trapping of the *ortho*-quinone **96** with ethyl vinyl ether 98
34. Synthesis of compound **121** 99
35. Chemical oxidation of catechols 100
36. Synthesis of compounds **133**, **134**, and **135** 102
37. Mechanism of the Schlosser modification 103
38. Synthesis of compound **131** 104
39. The use of TBDMS protecting groups 105

List of Abbreviations

AAME	aklanonic acid methyl ester
ACAT	acyl CoA:cholesterol *O*-acyl transferase
ACM	agar complete medium
ACP	acyl carrier protein
AK	aklaviketone
AMM	*Aspergillus* minimal medium
APT	attached proton test
AT	acyl transferase
ax	axial substituent
bp	boiling point
br	broad
BmT	(4*R*)-4-[(*E*)-butenyl]-4-methyl-*L*-threonine
Bu	butyl
BYADH	Baker's yeast alcohol dehydrogenase
Calc	calculated
CDI	carbonyl diimidazole
CFE	cell free extract
CI	chemical ionization
CoA	coenzyme A
COSY	correlated spectroscopy
δ	chemical shift in parts per million downfield from TMS
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DEBS	6-deoxyerythronolide B synthase
DEPT	distortionless enhancement by polarization transfer

DH	dehydratase
DIBAL-H	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
EI	electron impact ionization
eq	equatorial (substituent)
ER	enoyl reductase
ES	electron spray
FAB	fast atom bombardment
FAS	fatty acid synthase
FH	familial hypercholesterolemia
FM	fermentation medium
GM	growth medium
HDL	high density lipoprotein
HLADH	horse liver alcohol dehydrogenase
HMBC	heteronuclear multiple bond correlation
HMDS	hexamethyldisilazane
HMG-CoA	(3*S*)-hydroxy-3-methylglutaryl-coenzyme A
HMQC	heteronuclear multiple quantum coherence
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
IR	infrared spectroscopy
KR	ketoreductase
KS	ketosynthase
LDKS	lovastatin diketide synthase

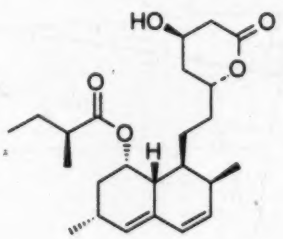
LDL	low density lipoprotein
LiHMDS	lithium hexamethyldisilazane
LNKS	lovastatin nonaketide synthase
LUMO	lowest unoccupied molecular orbital
m	multiplet (NMR), or medium (IR)
MCPBA	*m*-chloroperoxybenzoic acid
MeT	methyl transferase
mp	melting point
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
MS	mass spectrometry
NAC	*N*-acetylcysteamine
NAD	β-nicotinamide adenine dinucleotide
NADH	β-nicotinamide adenine dinucleotide, reduced form
NADPH	β-nicotinamide adenine dinucleotide phosphate, reduced form
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
ORF	open reading frame
OSC	oxidosqualene cyclase
p	pentet (quintet)
PABA	*p*-aminobenzoic acid
PKS	polyketide synthase
PLA_2	phospholipase A_2
PM	production media
Pyr	pyridine
R_f	retention factor
RM	replacement medium
RP	reverse phase

q	quartet
RNA	ribonucleic acid
r.t.	room temperature
s	singlet (NMR), or sharp (IR)
SAM	*S*-adenosyl-*L*-methionine
SE	squalene epoxidase
SM	seed medium
SS	squalene synthase
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TE	thioesterase
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultraviolet spectroscopy
VLDL	very low density lipoprotein
w	weak

INTRODUCTION

The goals of this thesis are to investigate the biosynthesis of lovastatin (**1**) (Figure 1) and to show that Diels-Alder reactions can be catalyzed enzymatically. Lovastatin (**1**) is an important cholesterol-lowering agent produced by fungi via a polyketide pathway potentially involving a biological Diels-Alder reaction. Other commercially available enzymes may also catalyze the cycloaddition of synthetic trienes.

Figure 1: Lovastatin (**1**)



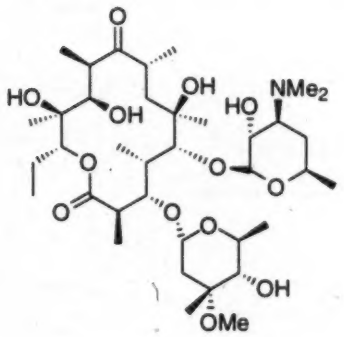
1

1. Biosynthesis of Polyketides: from Chemistry to Molecular Biology

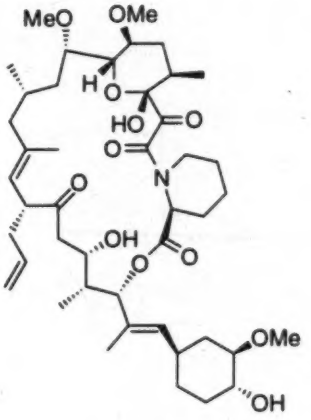
Polyketides[1] are a large family of complex natural products that are built from simple carboxylic acid monomers. Although the actual biological roles of these metabolites in the native producing organisms are often unclear, their interesting and versatile biological properties have made them useful pharmaceuticals, veterinary agents, and agrochemicals. Some selected examples include the antibiotics erythromycin A and tetracenomycin C, the immunosuppressants rapamycin and FK 506, the toxin aflatoxin B_1, and the antiparasitics monensin A (Figure 2). In spite of their structural diversity, most polyketides are thought to be assembled by a similar series of steps. Polyketide biosynthesis is related to fatty acid metabolism but differs, in that some of the reduction or dehydration reactions catalyzed by the polyketide synthase (PKS) can be suppressed at a

specific biosynthetic step. PKSs and fatty acid synthases (FASs) repeatedly condense simple carboxylic acid units (e.g. acetate or propionate) in a head-to-tail fashion. Whereas FASs usually reduce each acetate unit fully to give long aliphatic chains, PKSs selectively reduce each unit (acetate or other) to the oxidation level required, and selectively cyclize the molecule if necessary.

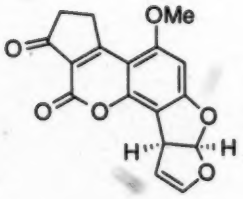
Figure 2: Examples of polyketides



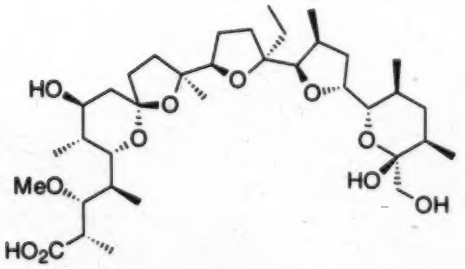
Erythromycin A



FK 506



Aflatoxin B_1

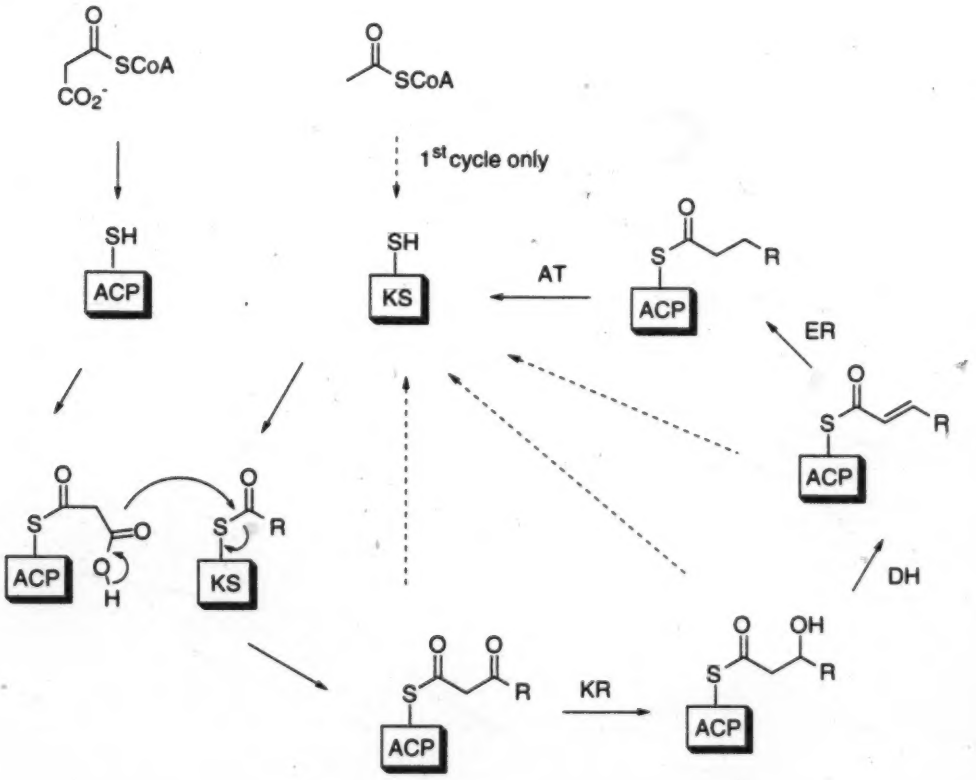


Monensin A

PKSs are exceptionally large multifunctional proteins.[1] The minimum PKS consists of four active sites each within separate domains: acyl carrier protein (ACP), ketosynthase (KS), and acyl transferase (AT). The active holoACP is formed by the

attachment of a 4'-phosphopantetheinyl group on a conserved serine residue of the inactive apoACP. This transformation by the phosphopantetheinyl transferase allows loading of the malonate or similar extender units or the growing acyl chain on the cysteamine thiol residue at the terminus of the phosphopantetheinyl group. In the first cycle (Figure 3), the starter unit,

Figure 3: Biosynthesis of polyketides

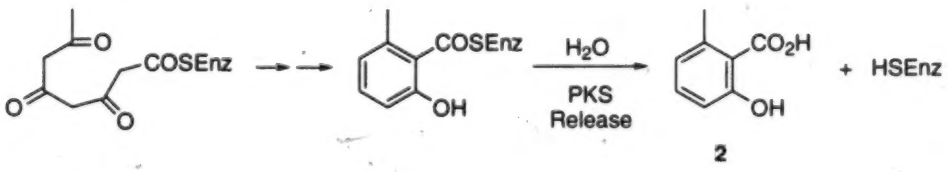


often acetyl CoA, is loaded on the KS, and the extender unit, malonyl CoA being the most common, is linked to the ACP. The decarboxylative condensation of the latter on the acetyl unit affords a β-ketoacyl-*S*-ACP. If necessary, the β-carbonyl unit is modified

by optional domains found on PKSs, such as ketoreductase (KR), dehydratase (DH), enoyl reductase (ER), and methyl transferase (MeT). After this first cycle, the diketide is transferred from the ACP to the active site cysteine of KS. Another malonate is then loaded onto the ACP, and condensed with the diketide to give a triketide. The β-carbonyl unit is again brought to the required oxidation level, and the cycle is repeated until the desired chain length is obtained. The intermediate oxidation states of the polyketide acyl chain generated at each cycle persist once the polyketide chain is transferred for further elongation.

When the polyketide has reached its full length, it is released from the enzyme, either by intermolecular attack by water or by intramolecular cyclization (assisted by the thioesterase (TE) in some cases), regenerating the free holo form of the ACP. The first termination route yields the free acid, as is the case in the biosynthesis of 6-methylsalicylic acid (**2**) by 6-methylsalicylic acid synthase (6-MSAS) (Figure 4).

Figure 4: Chain termination in the biosynthesis of 6-methylsalicylic acid (**2**)

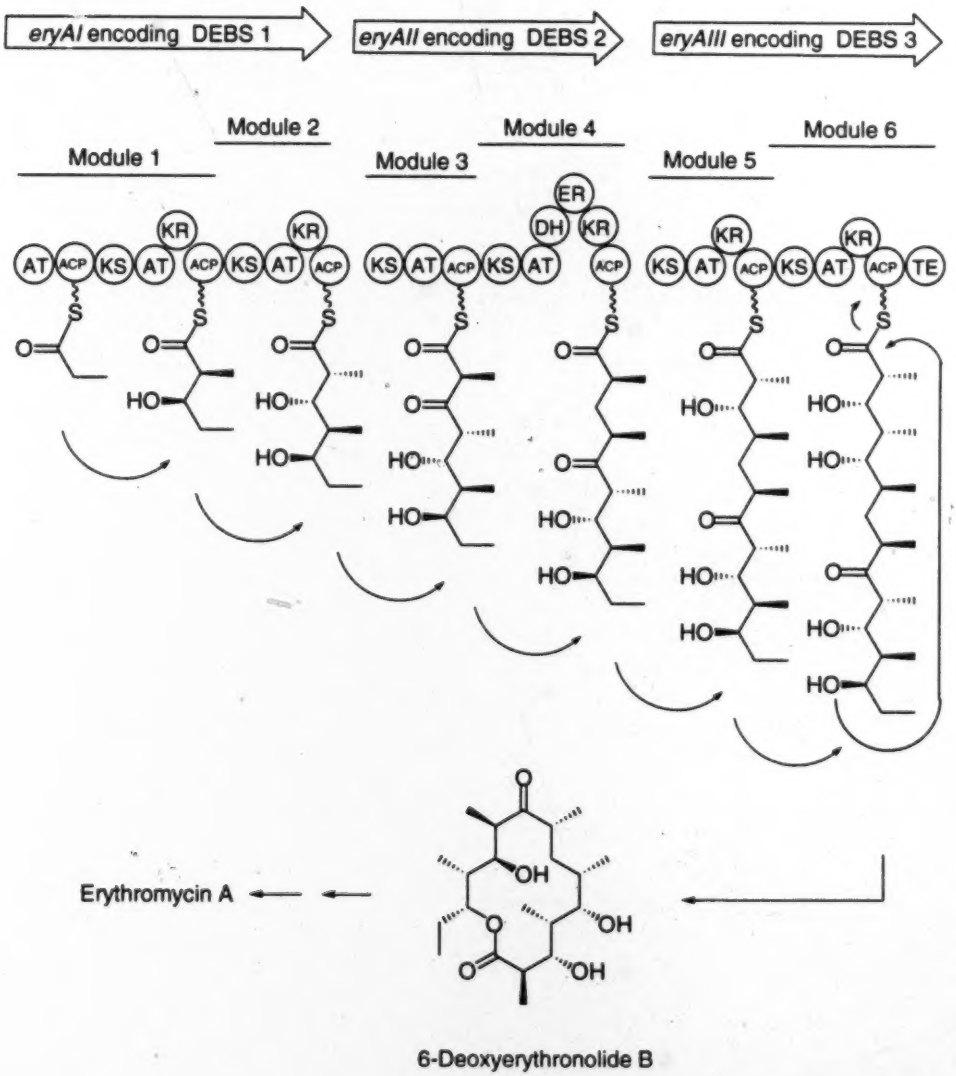


2

Alternatively, the intramolecular capture by a hydroxyl group present on the polyketide chain can result in the formation of a macrolactone, for example 6-deoxyerythronolide B, the PKS product leading to erythromycin (Figure 5). The PKS enzyme system that makes this metabolite, 6-deoxyerythronolide B synthase (DEBS) from *Saccharopolyspora erythraea*, is probably the most studied PKS at present. It consists of three large multifunctional proteins, DEBS1, DEBS2, and DEBS3, each

organized into two modules, with each module carrying the catalytic domains required to generate one extender unit. After release from the enzyme, the product is further

Figure 5: The biosynthesis of the polyketide portion of erythromycin, 6-deoxyerythronolide B, by deoxyerythronolide B synthase (DEBS). The different domains are represented as small circles.



processed by post-PKS enzymes to form erythromycin A. These tailoring enzymatic reactions include cytochrome P450 hydroxylation and glycosylation. Other common post-PKS transformations include alkylation (methylation, isoprenylation), dehydration, and epoxidation.

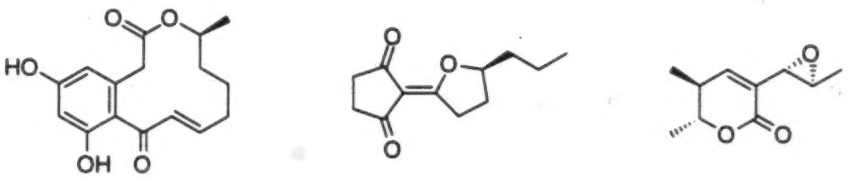
PKSs are classified according to the arrangement and use of their active sites.[1] Bacterial PKSs consist of either the modular type I or the iterative type II proteins, while fungal PKSs are iterative type I. Plant PKSs make up the type III. In the modular type I system, typified by DEBS, multiple catalytic sites are present in a large polypeptide chain and each is used only once to assemble a single molecule. The iterative type II system, like in the biosynthesis of tetracenomycin C, is constituted of separate polypeptides, each containing a single active site used as often as required. The iterative type I PKSs, for example 6-MSAS, contain one active site for each catalytic function, and these active sites are used repeatedly if necessary. In modular enzymes the final product can often be deduced from gene sequencing, however this is not the case for iterative systems. The factors determining when and how often each active site of the iterative PKSs are used remain undetermined.

Bacterial PKSs of both types (i.e. type I and type II) have been studied to a larger extent than fungal PKSs. Finally, the type III PKSs found in plants, such as chalcone synthases and stilbene synthases, are very different from all other known PKSs and FASs.[1g] Like other PKSs they catalyze the linking of two carbons units by repetitive decarboxylative condensations. However, there are major differences in the process, notably the participation of free CoA esters as substrates without the catalytic activity of the ACP. Protein sequence comparisons showed no significant overall resemblance with any PKSs of the other types, and a different motif surrounds the active-site cysteine. On the other hand, chalcone synthases and stilbene synthases share as much as 65% amino acid sequence identity, and build the same initial tetraketide from coumaroyl CoA.[1g] The type III superfamily also includes enzymes that generate tetraketides from other starter

units like isovaleryl CoA and isobutyryl CoA.[1g] Finally, plant PKSs that do not make chalcones have also been repoted.[1h]

Biosynthetic studies usually begin with a combination of techniques that include feeding of labeled precursors or accumulation of intermediates that are produced either by inhibition of specific enzyme activities or by mutated microorganisms. The latter can be generated with chemicals, X-rays, or UV.[2] Incorporation of advanced polyketide intermediates into intact cells is often successful in actinomycetes.[2] Successful incorporations of advanced intermediates is also possible with some fungal polyketides,[3] such as dehydrocurvularin (**3**) from *Alternaria cinerariae*,[4,5] oudenone from *Oudemansiella radicata*,[6] and aspyrone from *Aspergillus melleus* (Figure 6).[7]

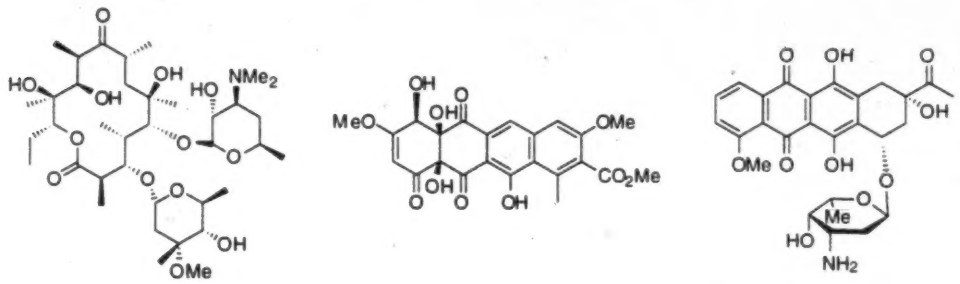
Figure 6: The fungal polyketides dehydrocurvularin (**3**), oudenone, and aspyrone



25 Oudenone Aspyrone

Cell free extracts (CFEs), in certain cases, also transform advanced intermediates into final bacterial polyketides. For example, cell free extracts of recombinant strains of *Streptomyces coelicolor* have been used to investigate the functional orientation of one of the AT domains involved during the biosynthesis of erythromycin A.[8] The gene cluster and the strict starter unit specificity of the tetracenomycin PKS system have been determined with cell free extracts of genetically engineered *S. glaucescens* strains.[9] Finally, the production of intermediates on the way to daunorubicin by cell free extracts of blocked mutants is restored by the addition of the appropriate genes.[10]

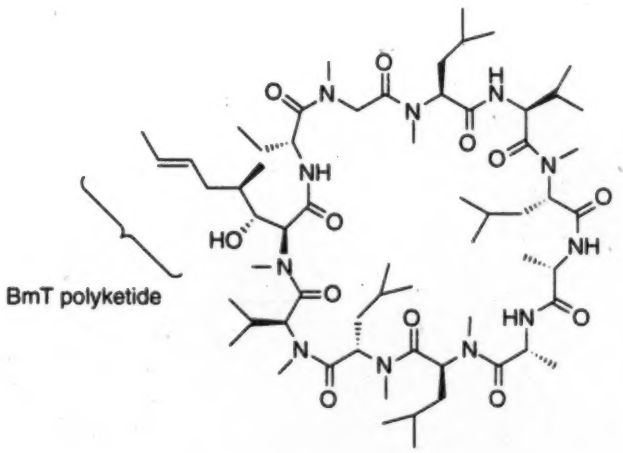
Figure 7: Erythromycin A, tetracenomycin C, and daunorubicin



Erythromycin A Tetracenomycin C Daunorubicin

The preparation of cell free extracts of *Penicilium patulum* and *Tolypocladium niveum* allows the intact incoporation of labeled intermediates into 6-methylsalicylic acid (**2**),[11] and into the polyketide portion-BmT-of cyclosporin A, respectively (Figure 8).[12]

Figure 8: The non-ribosomal peptide cyclosporin A contains the polyketide portion BmT ((4*R*)-4-[(*E*)-2-butenyl]-4-methyl-*L*-threonine)



Cyclosporin A

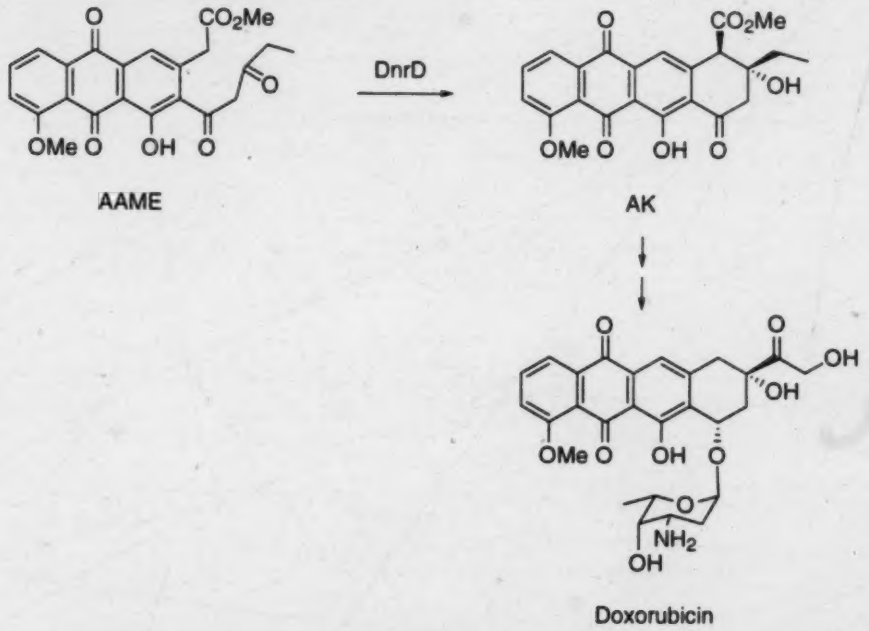
Over the past decade remarkable advances in microbial genetics redefined the field of biosynthesis and improved our understanding of the structures and mechanisms of

PKSs.[1] Four techniques commonly used for the elucidation of biosynthetic pathways and for the design of novel molecules include: 1) sequencing of the PKS genes and comparisons with protein databases to identify possible biochemical roles; 2) insertional inactivation of specific genes to generate mutants that accumulate intermediates in the pathway; 3) generation of deletion mutants through homologous recombination; and 4) expression of specific genes in a heterologous host to determine their function.

PKSs control the choice of starter unit, the nature and number of extender units, the degree of reduction and the stereochemistry of the product. A key challenge in delineating the mechanism of PKS enzymes is to determine how they are programmed to manage these variables. Significant advances with modular type I PKSs allow genetic manipulations that generate an array of novel "unnatural products", sometimes in a predictable fashion.[1] These manipulations include deletion of entire modules to reduce the chain length,[13] substitution of AT domains to alter starter and extender units,[14] and addition of domains (KR, DH, ER) to modules which naturally lack these activities.[15] The starter unit for some PKSs need not be acetate or propionate and can derive from metabolites in the shikimate pathway or from amino sugars.[16] The thioesterase domain on 6-deoxyerythronolide B synthase is responsible for the intramolecular cyclization (Figure 4). The configuration at a single stereocenter of the growing chain directs the cyclization either to a 14- or a 16-membered ring product.[17]

A number of type II polyketide cyclases such as ActVII,[18] DpsY,[19] TcmN,[20] and TcmI[21] are also known. The recent purification and characterization of the DnrD protein from the doxorubicin PKS system confirms its cyclase function.[22] The DnrD protein catalyzes the conversion of aklanonic acid methyl ester to aklaviketone, presumably *via* an intramolecular aldol condensation (Figure 9). Therefore, it appears that many type II PKSs employ a discrete enzyme to conduct the cyclization.

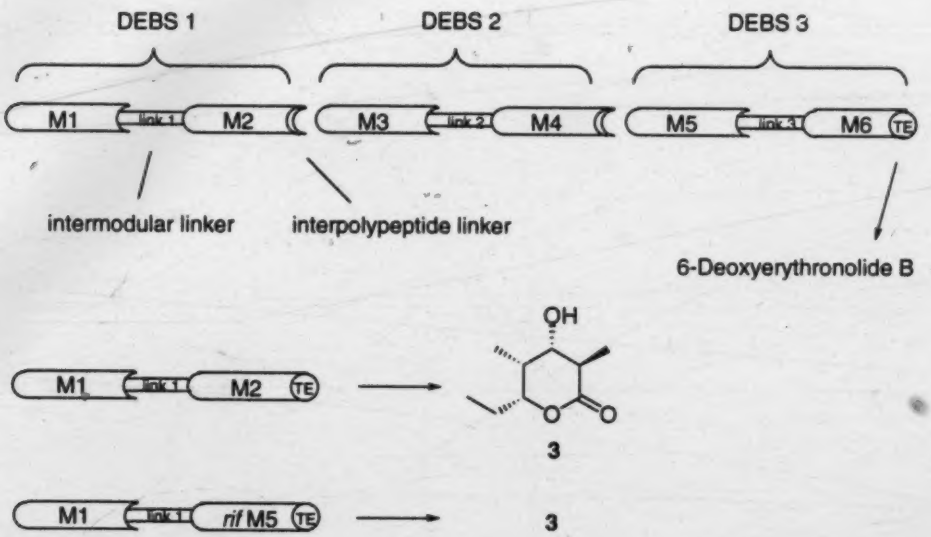
Figure 9: DnrD cyclization of aklanonic acid methyl ester (AAME) to aklaviketone (AK) during the biosynthesis of doxorubicin



One limitation to genetic manipulation of modular PKSs is that downstream modules may not efficiently process the anomalous product assembled by an engineered upstream module. Consequently, the gene engineering approach can result in decreased production of the target polyketide, and low yields are often observed. This problem may be solved by the recent discovery of intermodular communication in polyketide synthases by Khosla and coworkers.[23] Their results suggest that while the chemistry within PKS modules may be substrate- and stereo-selective, chain transfer between intact modules is permissive as long as the appropriate linkers can provide the connectivity between adjacent modules. These linkers are short segments of variable amino acid sequence. Intermodular linkers separate covalently connected modules and are characterized by the presence of relatively conserved proline residues. The longer hydrophilic interpolypeptide linkers are upstream of *N*-terminal modules and downstream of *C*-

terminal modules. For example, Gokhale *et al.*[23] have fused the module 1 (M1) of 6-deoxyerythronolide B synthase (DEBS) including the intermodular M1-M2 linker, with the M5 of rifamycin polyketide synthase (*rif* PKS) (Figure 10). The stereocenters generated by M5 of the *rif* PKS are the same as those generated by M2 of DEBS, but the incoming acyl chains differ considerably. The corresponding M1+*rif* M5+TE protein including the linker produced the same triketide lactone **3** and with comparable productivity as for the M1+M2+TE protein of DEBS. In addition to five other examples of successful use of intermodular linkers, Khosla and coworkers report one example containing an interpolypeptide linker.[23] Hence, it appears that perhaps the only requirement for communication between "unnatural" modules may be the presence of the appropriate linker.

Figure 10: Intermodular communication in polyketide synthases



The development of new genetic technologies also enables the preparation of "unnatural products" libraries by combinatorial biosynthesis.[24] The first reported creation

of a library of over 50 novel polyketides involved systematic mutations of DEBS.[24b] Using a single plasmid containing the genes encoding the three DEBS subunits, some domains were substituted by counterparts with different activities from the rapamycin PKS (*rap* PKS). The most productive single mutations in any module were combined to create double or triple mutants.

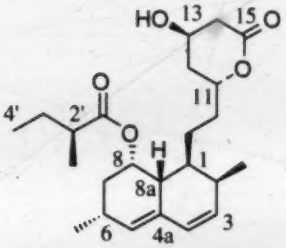
Shen *et al.* independently reported the production of a library of more than 30 polyketides by the expression of three PKS genes from the type II *whiE* PKS (spore pigment) of *Streptomyces coelicolor*.[24c] In their approach, the production of each polyketide requires a separate engineering step, whereas a more recent technology developed by Santi and coworkers allows rapid true combinatorial biosynthesis.[24a] The researchers found that a fully active DEBS is produced when the genes for each subunit are expressed separately from the same promoter. Their strategy involves the cloning of individual open reading frames (ORFs) on separate plasmids, followed by co-expression of selected ORFs in a suitable host to produce the PKS. Santi and coworkers prepared 14 vectors that were used to generate 64 triple transformants which all together produced 43 different polyketides, including 15 novel ones.[24a] Understanding fungal PKSs is critical to the implementation of these techniques to type I iterative systems for biological production of molecules with increased diversity.

2. Lovastatin and Related Cholesterol-Lowering Drugs

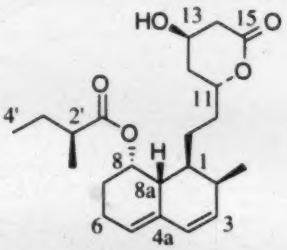
Lovastatin (**1**) (also known as mevinolin, monacolin K, or Mevacor™) and its derivatives are potent cholesterol-lowering drugs.[25] This fungal metabolite and its analog compactin (**5**) (Figure 11) were discovered because of their antifungal activity, and their competitive inhibition of (3*S*)-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34).[26,27] In 1976, Endo and his associates[28] isolated and characterized compactin (originally designated ML-236B) (**5**) from *Penicilium citrinum*.

This natural product is identical to the compactin independently isolated from *Penicilium aurantiogriseum* (originally termed *Penicilium brevicompactum*) by Brown *et al.*[29] Lovastatin (**1**) was first isolated in 1979 from *Monascus ruber* by Endo and coworkers[30] and from *Aspergillus terreus* by Merck researchers in 1980.[31]

Figure 11: Structures of lovastatin (**1**) and compactin (**5**)



1



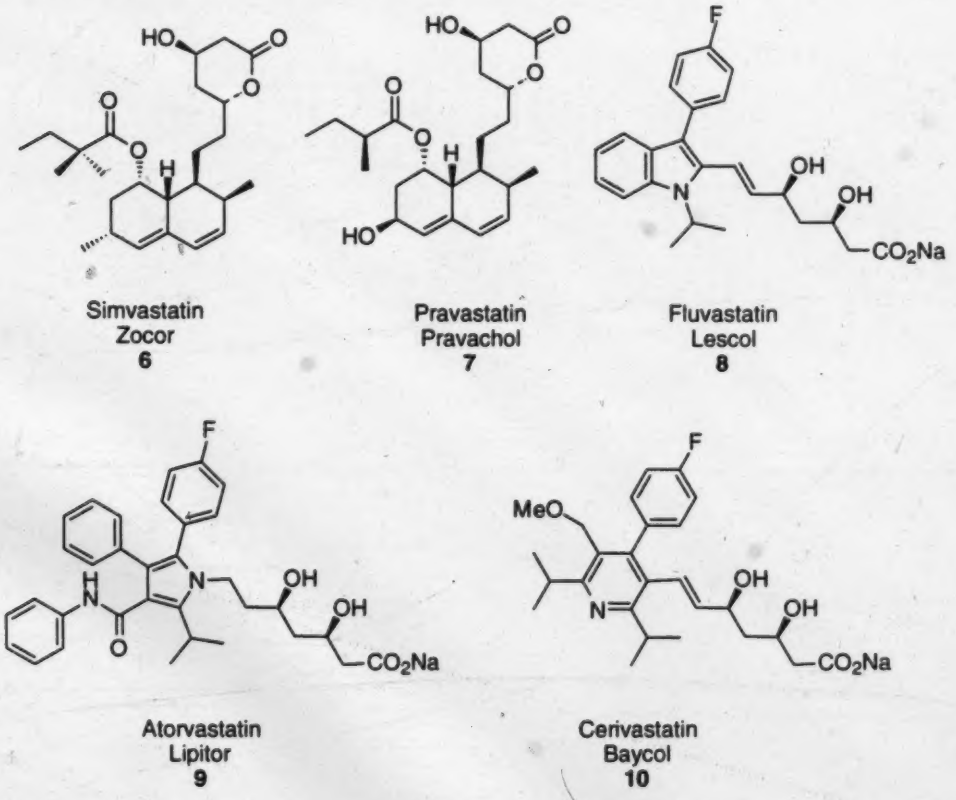
5

Different approaches have proven successful for the total synthesis of lovastatin (**1**)[32] and compactin (**5**),[33] as well as for the preparation of many derivatives.[34] Because all of these methods involve at least 20 steps, the isolation of lovastatin (**1**) from *A. terreus* remains the easiest route for commercial production. The syntheses of lovastatin derivatives and heterocyclic analogues[35] nevertheless provide key information for structure-activity relationships. For example, these studies show that the (3*R*)-3-hydroxy-δ-valerolactone moiety of lovastatin (**1**) is crucial for biological activity, whereas the highly substituted hexahydronaphthalene ring can be replaced by other lipophilic groups.[36]

Many other synthetic or semi-synthetic inhibitors of HMG-CoA reductase have since been discovered. In chronological order, the six inhibitors currently approved for use as drugs in Canada and in the United States are: lovastatin (**1**), simvastatin (**6**), pravastatin (**7**), fluvastatin (**8**), atorvastatin (**9**), and cerivastatin (**10**) (Figure 12).

Simvastatin (**6**) and pravastatin (**7**) are semi-synthetic drugs, and pravastatin (**7**) is also obtained by microbial transformation of compactin (**5**). On a mg/kg basis, cerivastatin (**10**) is the most potent HMG-CoA reductase inhibitor.[37] In enzyme inhibition tests based on the native ribosomal fraction isolated from rat liver, the IC_{50} values are: 1.1 nM for **10**, 66 nM for **6**, 77 nM for **1**, and 176 nM for **7**. Clinical studies indicated that **9** has a potency and efficacy higher than **6**.[37]

Figure 12: HMG-CoA reductase inhibitors

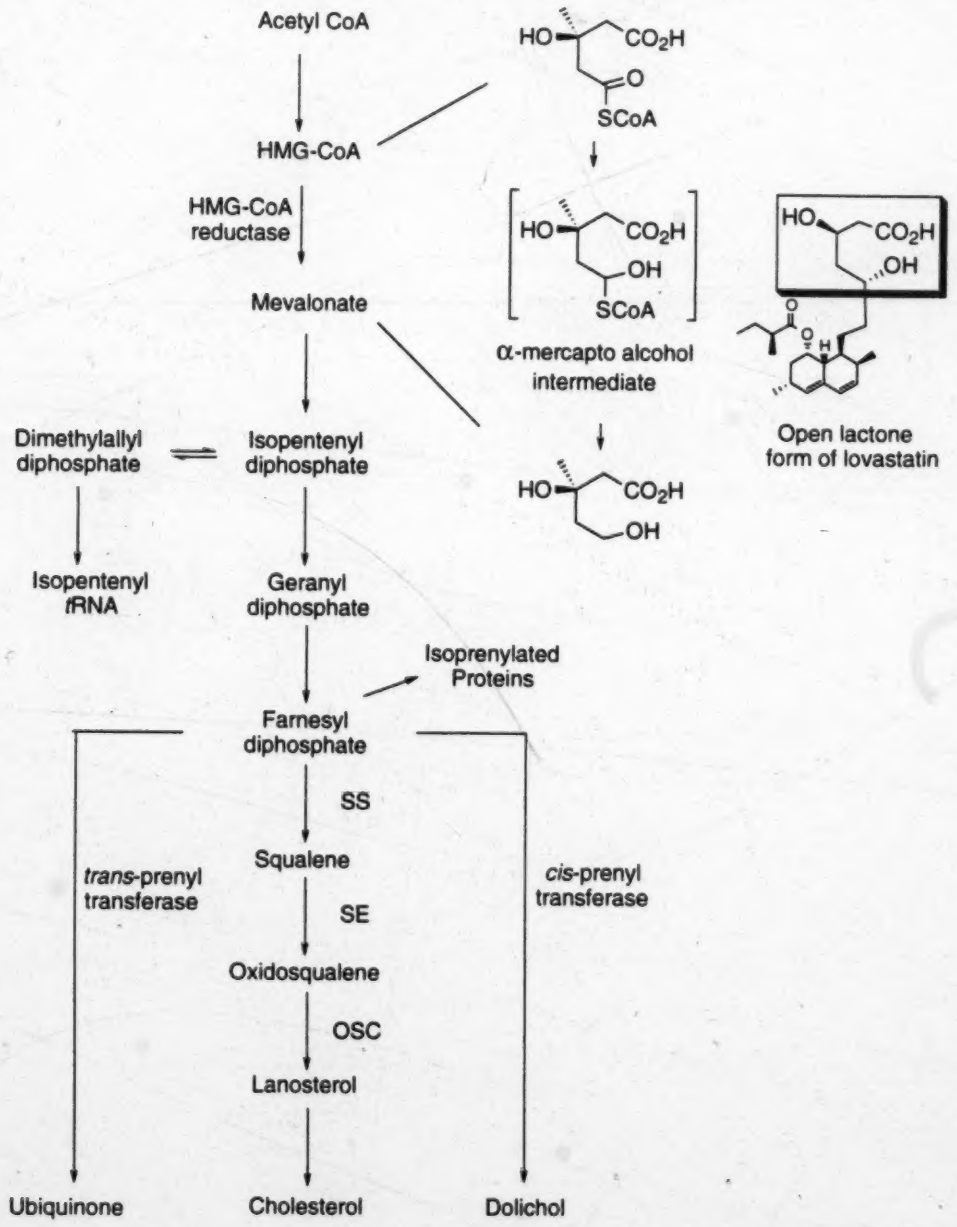


In humans, biosynthesis of cholesterol from acetyl CoA in the liver accounts for 60-70% of the total cholesterol pool,[37] making inhibition of this pathway an effective target for lipid-lowering therapy. Although their structures vary, all these HMG-CoA

reductase inhibitors have a mevalonate-like moiety in common. The rate limiting step in the biosynthesis of cholesterol is the transformation of HMG-CoA to mevalonate by HMG-CoA reductase (Figure 13). This process uses NADPH in two sequential reactions that proceed *via* an α-mercapto alcohol intermediate. Lovastatin (**1**) is administered as a prodrug, which converts into the corresponding hydroxy acid form[27] in the liver. This form is believed to mimic the proposed α-mercapto alcohol intermediate and interact simultaneously with both the HMG-CoA binding site and the adjacent hydrophobic pocket of the enzyme, thereby blocking the biosynthesis of cholesterol.[38] The resulting decrease in intracellular cholesterol concentrations promotes the expression of low density lipoprotein (LDL) receptors on the surface of hepatocytes, and results in an increased extraction of LDL cholesterol from the bloodstream.[39]

As an additional cholesterol lowering mechanism, HMG-CoA reductase inhibitors decrease the blood concentration of very low density lipoproteins (VLDL) and apolipoproteins, by inhibiting their synthesis and promoting their catabolism.[37] Because VLDL and apolipoproteins are precursors to LDL and triglycerides, the blood concentration of LDL is further decreased. Conversely, these drugs increase the concentration of favorable high density lipoprotein (HDL). Some other effects of the HMG-CoA reductase inhibitors include a 10% reduction of plasma viscosity, decreased platelet aggregation, and production of a relaxing effect on smooth muscles, potentially leading to reduction of blood pressure.[37] Even though these drugs are generally well tolerated, adverse effects include gastrointestinal disturbances, dyspepsia, myalgia, headache, sleep disorders, hepatomegaly, central nervous system disturbances, lens opacity, teratogenic and mutagenic effects.[37]

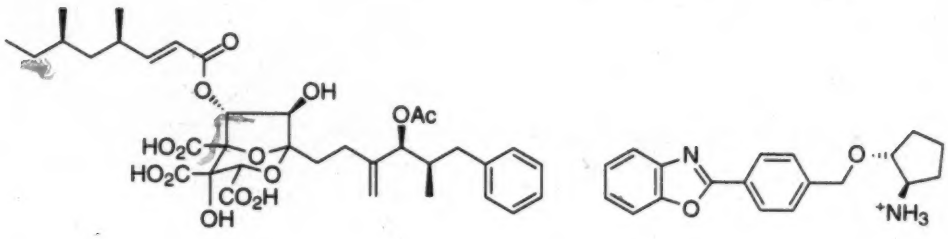
Figure 13: The biosynthesis of cholesterol and the mechanism of HMG-CoA reductase



HMG-CoA reductase inhibitors are, at the moment, the most effective hypocholesterolemic drugs.[37] As a result, extensive studies have been done to determine their metabolism and the factors causing their side effects.[37] Although some of the statins share close structural similarity, have similar mode of action, and similar toxicity profiles, analysis of their pharmacokinetics shows important differences. Their terminal half-life can vary from 30 min to 15 hours, and whereas pravastatin (**7**) is eliminated unchanged, the others are metabolized by various oxidations and/or alkylations. For example, the metabolism of lovastatin (**1**) leads to hydroxylation at C-6 and/or at C-3', or to the formation of an exomethylene at C-6.[37] In the case of fluvastatin (**8**), transformations other than oxidations and alkylations are also believed to occur, such as the cleavage of the aliphatic chain and/or the *N*-isopropyl group.[37] The metabolites of fluvastatin (**8**) do not contribute to the lipid-lowering activity, but the metabolism of the other statins produce compounds that retain their HMG-CoA reductase inhibitor activity.[37]

Because, HMG-CoA reductase inhibitors block the biosynthesis of cholesterol at an early stage, they are thought to inhibit the synthesis of other biologically important substances derived from mevalonate, such as ubiquinones, dolichols, isopentenyl tRNA and isoprenylated proteins.[40] A more selective inhibition of cholesterol biosynthesis may be achieved by inhibiting steps beyond farnesyl pyrophosphate, the common intermediate for all prenylation processes. Therefore the enzymes squalene synthase (SS), squalene epoxidase (SE), and oxidosqualene cyclase (OSC) (Figure 13) have also been targetted for hypolipidemic drug design.[41] Many inhibitors (e.g. zaragozic acid A and RPR 101821 for SS) have been found, but as of now, none have yet been approved for commercial use (Figure 14).

Figure 14: Inhibitors of squalene synthase (SS)



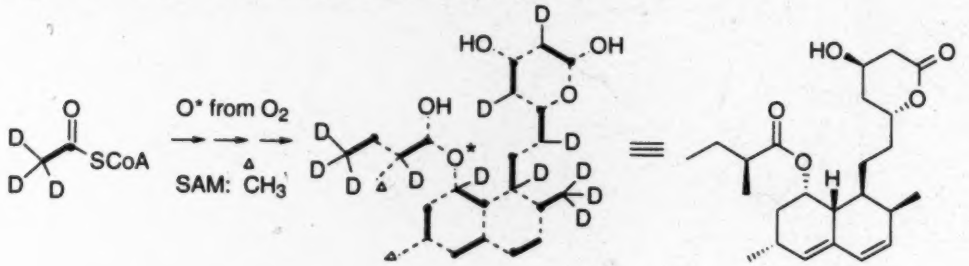
Zaragozic acid A
Squalestatin S1

RPR 101821

3. Biosynthesis of Lovastatin

Biosynthetic studies with fungi (e.g. *Aspergillus terreus*) show that lovastatin (**1**) is a polyketide derived from 11 acetate units, *S*-adenosyl-*L*-methionine (SAM), and oxygen (Figure 15).[42] In actinomycetes the methyl and ethyl side chains usually result from the incorporation of propionate and butyrate,[43] whereas in fungi the methyl side chains usually originate from *C*-methylation with SAM. This is the case in lovastatin (**1**), where both methyls at C-6 and at C-2' are derived from SAM.[42a] The oxygens at C-11, C-13, and C-15, initially believed to arise by aerobic oxidation,[42a] were re-examined using cultures of *A. terreus* MF 4845, and are in fact acetate derived.[42f] Additional acetate incorporation studies with *Penicilium aurantiogriseum* indicate that each atom of compactin (**5**) has the same biosynthetic origin as its equivalent one in lovastatin (**1**), indicating a similar assembly of both metabolites.[42f]

Figure 15: Origin of the atoms in lovastatin (**1**)

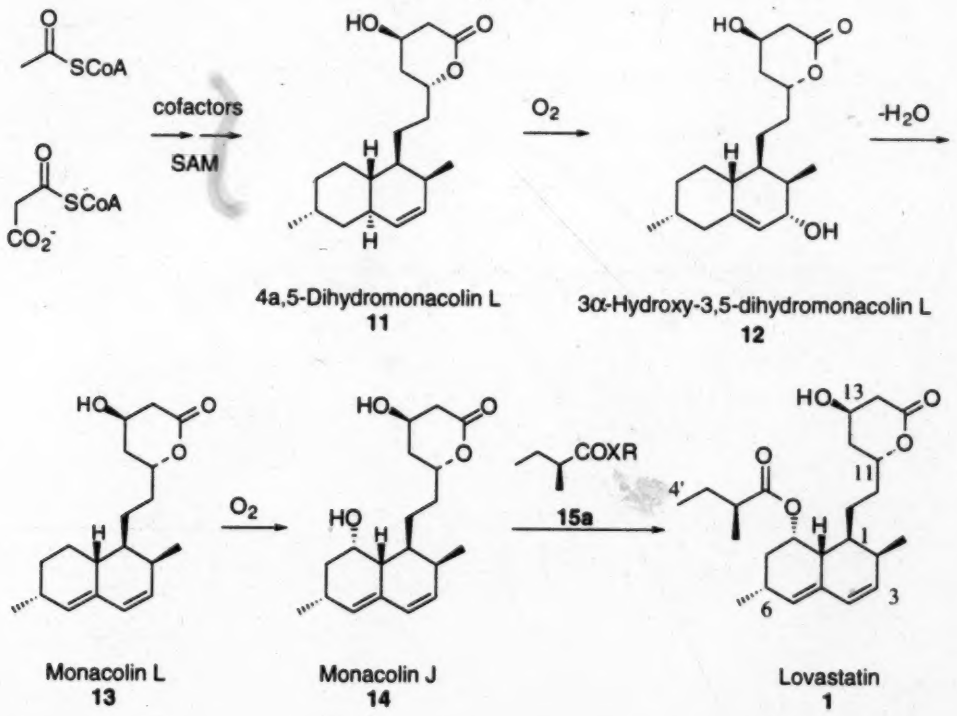


1

The labeling pattern of lovastatin (**1**) suggests that two polyketides, a nonaketide and a diketide, are initially formed by two different polyketide synthases: lovastatin nonaketide synthase (LNKS) and lovastatin diketide synthase (LDKS). The simplest isolated intermediate isolated in the pathway to lovastatin was the nonaketide 4a,5-dihydromonacolin L (**11**), which is further transformed into **1** through post-PKS modification steps that may proceed as proposed by Endo and coworkers (Scheme 1).[44]

Compound **11** was believed to be the first product released by the LNKS. Using cell free extracts of *M. ruber*, Endo and his associates have demonstrated the transformation of 4a,5-dihydromonacolin L **11** to 3α-hydroxy-3,5-dihydromonacolin L (**12**).[44a] They believed that the unstable compound **12** spontaneously dehydrates to give monacolin L (**13**), but researchers at Merck Sharp & Dohme Research Laboratories concluded that **13** is formed from **12** only as an artifact of isolation rather than as a discrete product of biosynthesis in *A. terreus*.[45] This is supported by the fact that no monacolin L (**13**) can be detected in freshly harvested *A. terreus* cultures, however an increasing amount appears during the isolation of lovastatin (**1**). Hydroxylation at C-8 of monacolin L (**13**) by molecular oxygen present in the cell-free system of Endo and coworkers provides monacolin J (**14**).[44e] This nonaketide is converted to lovastatin (**1**) in the cell free extract by the esterification with the diketide (2*R*)-methylbutyric acid (**15**).[44b]

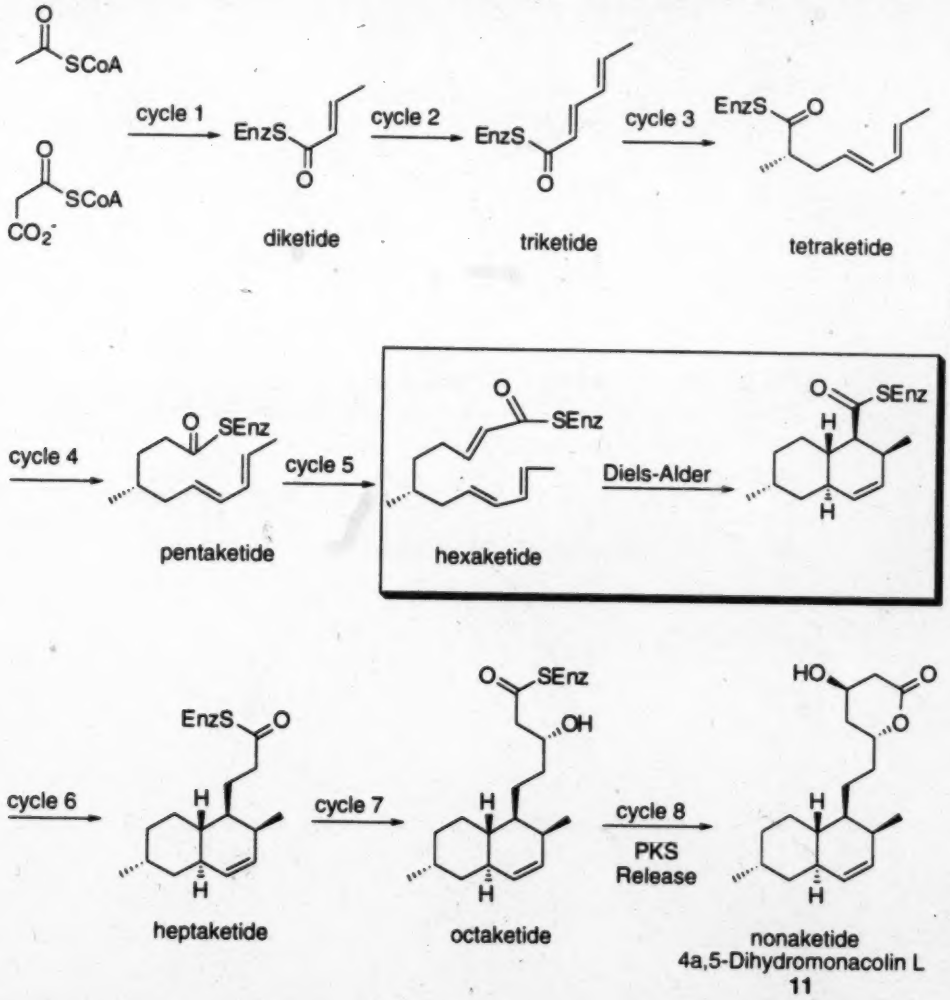
Scheme 1: Post-PKS transformations leading to lovastatin (**1**)



At that stage, the details of the assembly of **11** (i.e. the mechanism of LNKS) were unknown.

The current theories on polyketide biosynthesis and the labeling pattern observed for lovastatin (**1**) allow the following pathway for the assembly of 4a,5-dihydromonacolin L (**11**) to be proposed (Scheme 2).[42a,42e,42f,46] Condensation of acetate and malonate followed by reduction and dehydration produces a diketide intermediate.

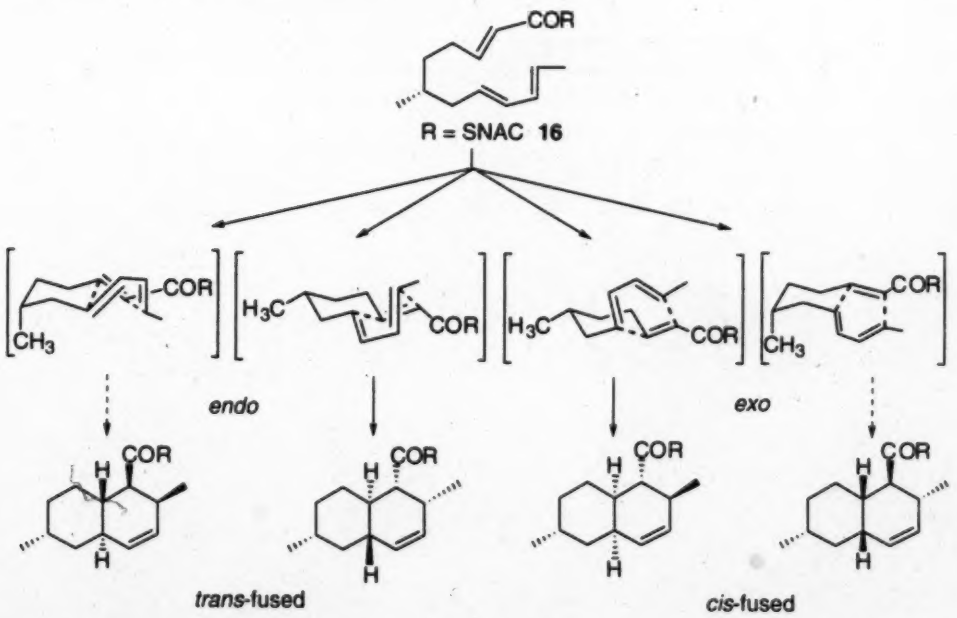
Scheme 2: Proposed pathway for the biosynthesis of 4a,5-dihydromonacolin L (**11**)



A second malonate is added, reduced and dehydrated to give a triketide. This process is then repeated in cycles with the appropriate transformations at each step performed by KR, DH, ER, or MeT if necessary, leading to the hexaketide. The putative hexaketide could undergo a Diels-Alder reaction, as suggested by the stereochemistry of 4a,5-dihydromonacolin L (**11**). The resulting bicyclic adduct could then further be extended as before to the nonaketide **11**.

The chemical Diels-Alder reaction of **16**, the *N*-acetylcysteamine thioester (RCOSNAC) of the hexaketide proposed for the biosynthesis of lovastatin (**1**), has been shown to proceed *via* chair-like *exo* and *endo* transition states with the methyl substituent pseudoequatorial (Scheme 3).[46] The putative biosynthetic Diels-Alder cycloaddition

Scheme 3: The four possible diastereomeric products resulting from the Diels-Alder reaction of the postulated intermediate triene



leading to lovastatin would require an *endo* conformation, but with the methyl substituent pseudoaxial.[46]

4. Recent Advances in Chemical and Enzymatic Diels-Alder Reactions

One of the most interesting features of the proposed biosynthetic pathway to lovastatin (**1**) is the possible occurrence of an enzyme-catalyzed Diels-Alder reaction. Since Otto Diels and Kurt Alder were awarded the 1950 Nobel prize in chemistry, the

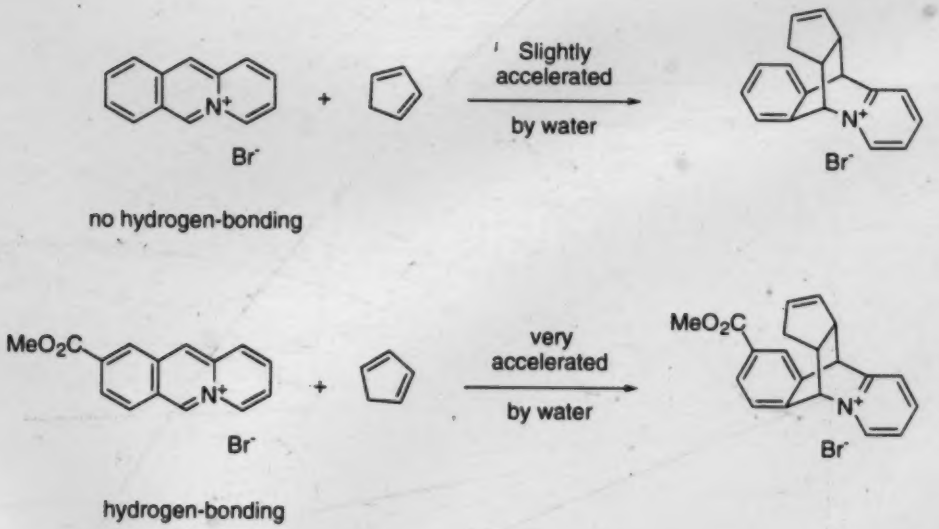
reaction bearing their names is one of the most widely used synthetic procedures, with more than a dozen articles published each week.[47] This [4+2] cycloaddition reaction is concerted with both new bonds formed in the same transition state, but is not necessarily synchronous.[48] The *endo* pathway is often preferred over the *exo* one because secondary orbital interactions stabilize the transition state.[48] The reaction is facilitated by electron-rich dienes and electron poor dienophiles (normal electron demand), but the opposite arrangement wherein electron-poor dienes react with electron rich dienophiles (inverse electron demand) is also favored. For a normal electron demand Diels-Alder reaction, the rate and selectivity depend on the efficiency of the interaction between the HOMO of the diene and the LUMO of the dienophile. Raising the energy of diene HOMO and lowering the energy of the dienophile LUMO increases the mutual overlap. Therefore, most dienophiles carry an electron-withdrawing substituent to lower their LUMO energy. In addition to the retro-Diels-Alder reactions[49] and the traditional intermolecular Diels-Alder cycloadditions,[50] the intramolecular[47] variants have allowed syntheses of numerous natural products. The intramolecular [4+2] process permits simultaneous construction of two or more rings and often offers improved regio- and stereochemical control by limiting the flexibility of the system. The use of tethers,[51] or temporary connections, transforms a potential intermolecular reaction into an intramolecular one. Because intramolecular Diels-Alder reactions are more entropically favored than their counterparts, the use of tethers can enable or accelerate the reactions between otherwise unreactive components. There are several criteria for efficiency in this strategy. The tether attachment and cleavage reactions must be efficient. The nature of the linker must not disfavor the close approach of the diene and dienophile necessary for the cycloaddition to take place. Ideally, tethers that provide a more rigid triene should afford improved regio- and stereoselectivity, although prediction of the resulting isomer ratio remains challenging. Craig and coworkers reported the successful use of tethers (carbon and silyl acetals, benzylic and tertiary ethers, and diesters) in the Diels-Alder reactions between 2,4-hexadienol

derivatives and 4-hydroxy-2-butenoate derivatives.[52] The diphenylsilyl or diester tethered trienes afforded preferentially the *cis*-fused *exo* bicyclic products. More recently, Batey and coworkers connected alkenic boronic esters to dienes to give eight alkenyl boronic ester trienes by the standard hydroboration from the corresponding alkynes.[53] After the cycloaddition, which gave the *endo* product as the predominant diastereoisomer for all of them, the tethers were easily removed by treatment with trimethylamine-*N*-oxide followed by hydrolysis.

Among the procedures developed to increase the yield and selectivity of Diels-Alder reactions,[54] the discovery that Lewis acids can catalyse this process has had a large impact on synthetic organic chemistry.[55] The coordination of a Lewis acid to the electron-poor dienophile leads to further enhancement of its electron-withdrawing capacity and lowering of the LUMO energy, thereby increasing the rate and selectivity. Water can improve selectivity and accelerate[56] Diels-Alder reactions up to 12800 times.[57] Aqueous media are believed to favor the more polar and compact *endo* activated complex by a combination of two factors. The low affinity of the reactants for water, compared to organic solvents, forces them into close contact, leading to a reduction of the molecular surface area exposed to water. This effect is called "enforced hydrophobic interactions".[56] Since Diels-Alder reactions are characterized by a decrease of the surface area going from the initial state to the transition state, enforced hydrophobic interactions lower the energy of activation, and the reaction is accelerated. The second effect results from hydrogen bonding of water to the activating group of the dienophile and lowers the energy of activation in the same way as Lewis acids. These proposals are supported by computer simulations.[58] The separation and quantification of both factors can be achieved with molecules that favor one of the two effects. For example, to show that an important part of the observed rate accelerations could be attributed to hydrogen bonding, the dimerization of cyclopentadiene, in which hydrogen bonding is excluded, was examined.[59] Although rates were measured, the calculations were complicated by the

high volatility of cyclopentadiene, and thus the findings remain disputed.[59] However, the reaction of acridizinium bromide with cyclopentadiene wherein the hydrogen-bond donor and acceptor groups are absent or very weak (Figure 16),[60] show only modest rate

Figure 16: Effects involved in the acceleration of the Diels-Alder reaction by water



accelerations in water-rich media. In contrast, the cycloaddition of cyclopentadiene with 9-carbomethoxyacridizinium bromide, which does contain a hydrogen-bond acceptor, gives a substantial rate acceleration in aqueous media. Retro-Diels-Alder reactions were used by Engberts and coworkers because of the negligible contributions of hydrophobic interactions, thus providing an opportunity to quantify the influence of hydrogen bonding alone on cycloadditions.[61] The forward reaction of nitrosobenzene with cyclopentadiene is greatly accelerated by water, while the reverse reaction is retarded. In contrast, the retro-Diels-Alder reaction of anthracene dione is faster in aqueous solutions. The efficient hydrogen-bond-donating ability of water appears to govern the increased reactivity by stabilizing the polarized activated complex, despite the negligible hydrophobic effects.

Presently, it appears that many types of reaction benefit from an aqueous reaction medium, including Claisen rearrangements,[62] aldol condensations,[63] benzoin condensations,[64] ene reactions,[65] and 1,3-dipolar cycloadditions.[66]

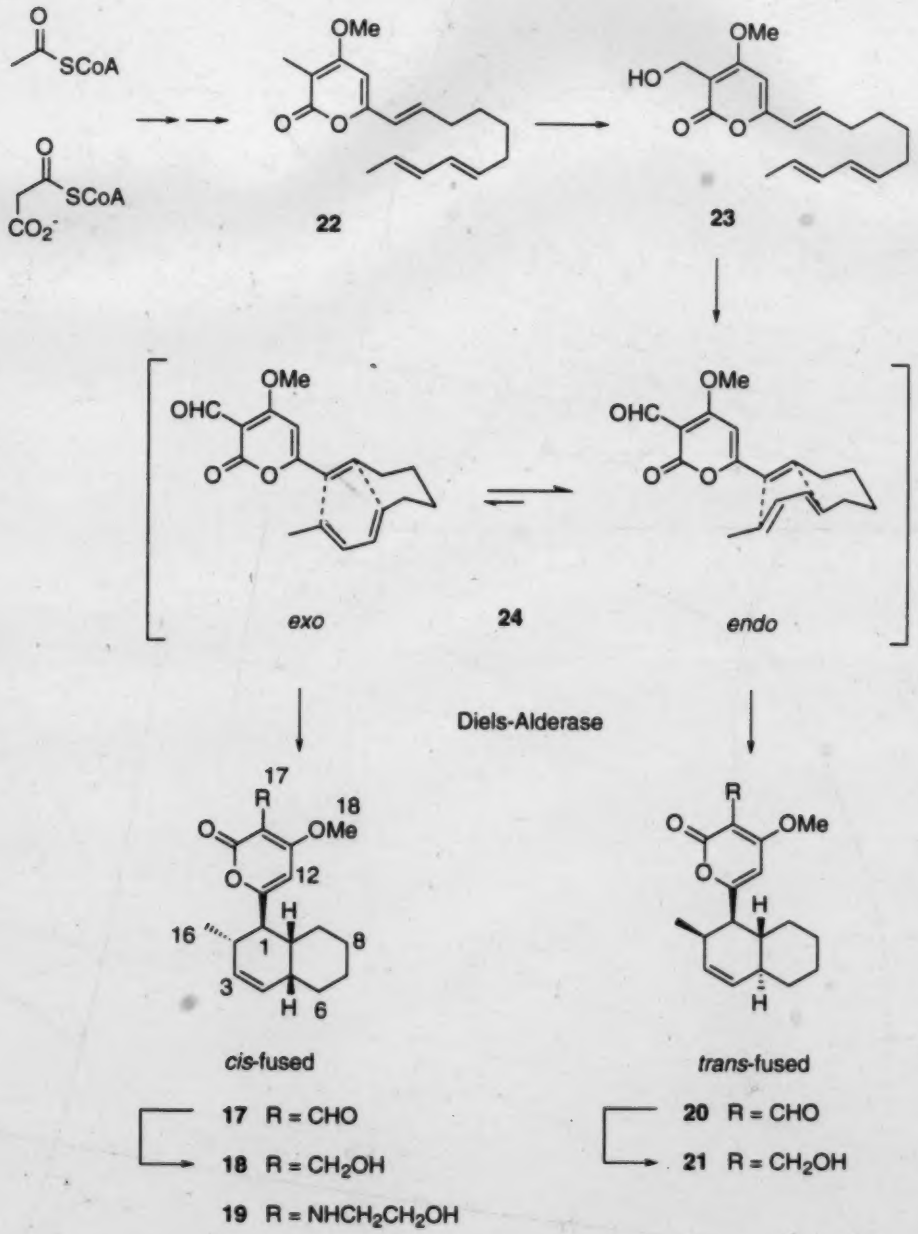
The beneficial effects of Lewis acids and water can be successfully combined to achieve even greater rate accelerations.[67] The effect of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} ions on the rate and *endo-exo* selectivity have been studied for five different dienophiles with cyclopentadiene. The catalysis by 0.001 M $Cu(NO_3)_2$ in water accelerates the reaction by a factor of 79300 relative to the uncatalyzed reaction in acetonitrile. The increase of *endo-exo* selectivity induced by water alone, is completely suppressed when the Lewis acid is added to the aqueous reaction mixture. The addition of micelles like sodium dodecylsulfate, cetyltrimethylammonium bromide, copper, or zinc didodecyl sulfate, to this system afforded a million-fold acceleration compared to the uncatalyzed reaction in acetonitrile. Lately, Zhu *et al.* have used methylrhenium trioxide as a Diels-Alder catalyst in aqueous medium with success.[68]

During the last two decades, the development of new and highly enantioselective processes has become a major focus for synthetic chemists, and the use of chiral catalysts is displacing chiral reagents and auxiliaries as the method of choice for asymmetric synthesis. An efficient chiral catalyst allows for large quantities of optically active product to be obtained using relatively small amounts of inexpensive enantiopure material. Since the Diels-Alder reaction introduces up to four new asymmetric centers in one step, great effort has been devoted to the elaboration of catalytic enantioselective versions of this reaction. The chiral catalysts often include metals like aluminum, boron, copper, iron, nickel, or titanium, held by chiral groups such as binaphthols or bisoxazolines.[69] Chiral catalysts prepared from lanthanides, like ytterbium triflate,[70] have also been used. Finally, some water-tolerant chiral catalysts have also been prepared.[71] In addition, Diels-Alder reactions have been promoted by many other agents like microwaves,[72] ultrasound,[73] supercritical water,[74] ionic liquids,[75] organic solids (solid state),[76] addition

of salts (e.g. $LiClO_4$)[77] or surfactants,[78] molecular encapsulation,[79] bovine serum albumin,[80] antibodies,[81] or RNA.[82]

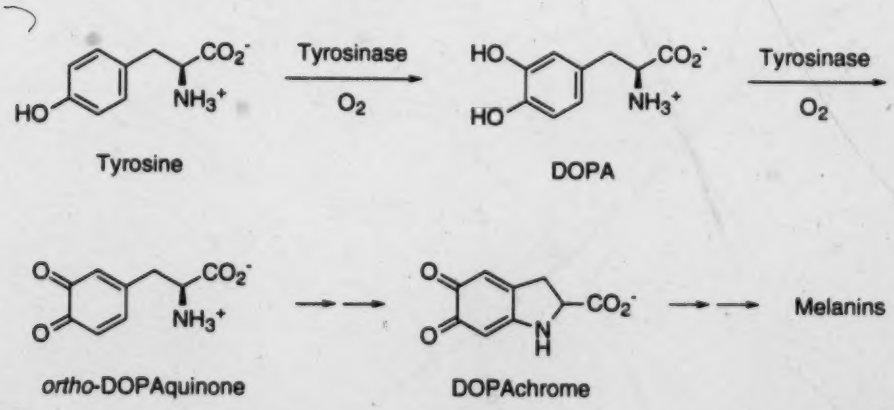
Enzymes represent another group of potential catalysts for Diels-Alder reactions. Although numerous secondary metabolites now are proposed to be biosynthesized via an enzymatic Diels-Alder reaction,[83] this has been experimentally proven only for two cases: solanapyrones,[84] and lovastatin (work presented in this thesis). Solanapyrones A (**17**), B (**18**), C (**19**), D (**20**), and E (**21**) (Scheme 4) are phytotoxins produced by *Alternaria solani* and *Ascochyta rabiei*, causative agents of tomato and potato early blight, and chick pea blight diseases, respectively. Their structures were elucidated by spectroscopic methods and chemical degradation,[85] and confirmed by total synthesis[86] and X-ray analysis.[87] Biosynthetic studies of solanapyrones show that the carbon skeleton is constructed by a polyketide pathway from one acetate, seven malonate and two C_1-units from SAM.[88] The co-occurence of diastereomeric adducts **17** and **20** strongly suggests that they might be produced by way of a Diels-Alder reaction from the corresponding achiral triene precursor. The first direct evidence of such a biological reaction came in 1994 from *in vivo* incorporation studies with *A. solani* by Oikawa *et al.*[89] Intact incorporation of the acyclic labeled trienes prosolanapyrone I (**22**) and prosolanapyrone II (**23**) into **17** and **20** occurs with incorporation rates of 0.26 and 0.37% respectively. In order to eliminate the possibility of a nonenzymatic cyclization, the labelled **17** was converted to its MTPA ester and shown to have an optical purity of ca. 99%. The involvement of a Diels-Alderase in the biosynthesis of solanapyrones was later confirmed with a crude enzyme extract.[90] The chemical [4+2] reaction of prosolanopyrone III (**24**) both under organic and aqueous conditions (30 °C and 10 min) gives a 3:97 *exo:endo* mixture. Under the same conditions, no cycloadduct is detected with the alcohol prosolanapyrone II (**23**). A cell free extract of *A. solani* converts **24** to **17** and **20** with a 87:13 *exo:endo* ratio. When the enzymatic solution is concentrated four times, the

Scheme 4: The biosynthesis of solanapyrones involves an enzyme-catalyzed Diels-Alder reaction



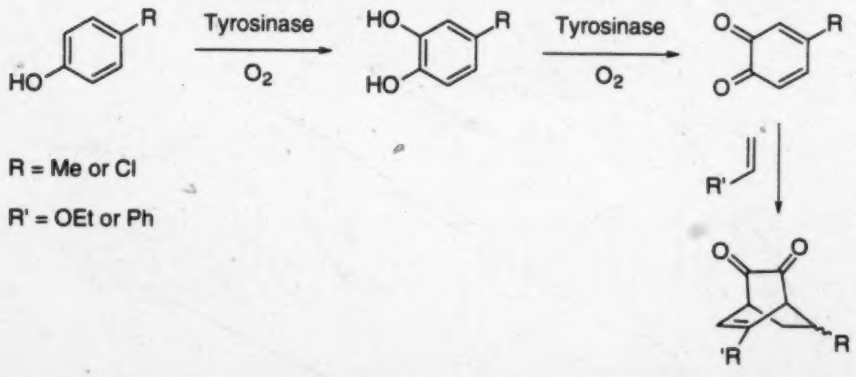
reaction proceeds 4.1 times faster than the non-catalyzed thermal process. The crude enzyme also converted the precursor alcohol **23** to **17** and **20** via a Diels-Alder reaction with the same 87:13 *exo-endo* ratio. The acceleration of the reaction rate and the stereoselectivity suggest that the cycloaddition of **24** to **17** is catalyzed by a specific enzyme. The total suppression of cycloaddition under anaerobic conditions indicates that the conversion of **23** to **24** is not a dehydrogenation but an oxidation. Finally, solanapyrone synthase was partially purified, and it appears that both the oxidation and the Diels-Alder reactions may be catalyzed by a single enzyme. The results suggest that enzymatic hydroxylation of the hydroxymethyl group of **23** affords an aldehyde hydrate that converts to the more reactive dienophile **24**. This presumably cyclizes in the active site where it is held in the conformation leading to *exo* product. Another type of oxidative enzyme-triggered Diels-Alder reaction is known. Tyrosinase (EC 1.14.18.1) catalyzes the aerobic oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) with subsequent further oxidation to *ortho*-DOPAquinone which is a key step in the biosynthesis of many natural pigments (Scheme 5).[91] Waldmann and coworker developed an ingenious synthesis of highly functionalized bicyclic 1,2-diketones

Scheme 5: Reactions catalyzed by tyrosinase



involving a tyrosinase-initiated hydroxylation-oxidation-Diels-Alder reaction cascade (Scheme 6).[92] Mushroom tyrosinase was immobilized on glass

Scheme 6: Enzyme-initiated hydroxylation-oxidation-Diels-Alder reaction cascade



beads and used in chloroform to catalyze the oxidation of phenols to the corresponding *ortho*-quinones.[92] Aqueous conditions could not be used because they lead to the polymerization of *ortho*-quinones which in turn inactivate the enzyme. When this enzymatic reaction was performed in presence of ethyl vinyl ether or vinyl benzene, the *ortho*-quinones produced *in situ* were trapped by the dienophiles via a Diels-Alder reaction (Scheme 6). The stereochemical outcome of the cascade reaction yielded racemic products, which suggested that although tyrosinase did catalyze the oxidation of the phenols, the intermolecular cycloadditions were not catalyzed by the enzyme.

The goals of this project were to investigate the biosynthesis of lovastatin (**1**) and to show that Diels-Alder reactions can be catalyzed by enzymes. Biosynthetic tools such as *in vivo* and *in vitro* incorporation of labeled intermediates using wild type and mutated fungal strains were envisioned. Purified lovastatin PKS can be reacted with the proposed hexaketide intermediate **16**, or with other trienes. Finally, commercially available enzymes may catalyze the stereoselective cycloaddition of synthetic compounds.

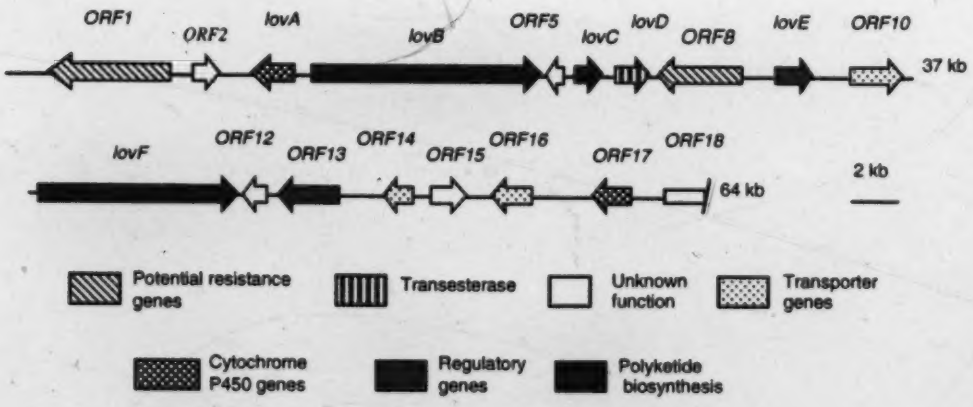
RESULTS AND DISCUSSION

1. Molecular Biology of Lovastatin Biosynthesis

1.1 Sequencing of the lovastatin gene cluster by Professor Hutchinson at the University of Wisconsin

Since secondary metabolism genes are normally clustered in microorganisms, once the lovastatin nonaketide polyketide synthase (LNKS) gene was identified,[93] it became potentially possible to explore the surrounding gene cluster with molecular biology techniques. Professor C. R. Hutchinson and his coworkers at the University of Wisconsin undertook this as part of a collaborative effort with our group. Eighteen putative lovastatin biosynthetic genes could be located, and functions could be tentatively assigned for thirteen of them based on comparison with databases of known proteins (Figure 17 and Table 1).[94]

Figure 17: Lovastatin gene cluster*



* This figure has been generously provided by Professor Hutchinson at the University of Wisconsin. It has been slightly modified.

Table 1: Proposed proteins encoded by the genes present in the biosynthesis cluster of lovastatin*

Gene name	Proposed function	Predicted molecular weight of protein (Da)
ORF1	Possible role in resistance	170,495
ORF2	Not known	37,094
lovA	Cytochrome P450 - Hydroxylation	60,589
lovB	Biosynthesis of nonaketide	334,983
ORF5	Reductive step	28,979
lovC	Biosynthesis of nonaketide	39,508
lovD	Transesterase	46,034
ORF8	Self resistance	114,981
lovE	Positive regulatory gene	55,424
ORF10	Transport of lovastatin or resistance	57,589
lovF	Biosynthesis of diketide side chain	276,621
ORF12	not known	27,845
ORF13	Regulation of lovastatin biosynthesis	83,328
ORF14	Precursor supply for lovastatin biosynthesis	32,106
ORF15	not known	52,847
ORF16	not known	52,488
ORF17	Cytochrome P450 - hydroxylation	58,576
ORF18	not known	>53,356

* This table has been generously provided by Professor Hutchinson at the University of Wisconsin.

As expected, two PKS encoding genes occur in the cluster, as well as an esterase encoding gene (*lovD*), which is important in linking both polyketides, monacolin J (**14**) and (2*R*)-methylbutyrate (**15a**), together (see Scheme 1). The six domains characteristic of PKSs (KS, AT, DH, ER, KR, ACP) plus a methyl transferase (MeT) domain are observed for both LNKS (*lovB*) and lovastatin diketide synthase (LDKS, *lovF*). LNKS

does not contain a obvious thioesterase (TE) active site, but it has a *C*-terminal condensation domain similar to a non-ribosomal peptide synthase cyclization domain. Unlike LDKS, the LNKS has an ER domain that does not conform well to the consensus sequence for such regions in FASs and many PKSs. Consequently, it may be inactive. Two genes (*lovA* and ORF17) believed to be involved in the post-PKS biosynthetic steps have been assigned as cytochrome P450 proteins based on sequence similarities to known enzymes. Sequencing also suggests that two genes (ORF1 and ORF8) may be involved in resistance, and two (*lovE* and ORF13) in the regulation of lovastatin (**1**) production. The latter has allowed generation of *A. terreus lovE* strain that overproduces lovastatin (**1**).

1.2 Genetically engineered fungal strains prepared by Professor Hutchinson at the University of Wisconsin

Except for *Penicillium aurantiogriseum*, the different fungal strains used in the present studies are mutants prepared at the University of Wisconsin by the research group of Professor Hutchinson. Three key techniques have been used by our collaborators:[94] insertional inactivation, preparation of deletion mutants, and the expression of certain genes in a heterologous host, *Aspergillus nidulans*. The organisms used in our work are listed in Table 2. They have been assigned UAMH numbers from the University of Alberta Microfungus Collection and Herbarium where they are stored, as well as WMH numbers from Professor Hutchinson and his coworkers as described in the literature.[94]

Table 2: Genetically engineered fungal strains used in this work

Organism	UAMH & WMH	Description[94]
A. terreus control	9156	phleomycin resistance vector added to the wild type
A. nidulans control (GM32.4)	8966	plasmid with a marker coding for the biosynthesis of uridine added to a strain that requires PABA and uracil
A. terreus lovE	9155	contains extra copies of *lovE* under the control of the starch-inducible *A. oryzae* glucoamylase promoter and a phleomycin resistance marker
A. terreus XS-1P	9271	contains extra copies of *lovE* under the control of its own promoter and a phleomycin resistance marker
A. nidulans lovB (TPKS-F)	8965 1738	plasmid inserted in the chromosome containing the uridine marker and *lovB* under the control of the ethanol/cyclopentanone-inducible *alcA* promoter
A. terreus lovA (P450I)	9297	the *lovA* gene of *A. terreus* control was inactivated (function inferred from sequence similarities)
A. terreus lovC (dehydrogenase)	9298 1734	the *lovC* gene of *A. terreus* control was inactivated (function inferred from sequence similarities)
A. terreus lovD (esterase)	9295 1732	the *lovD* gene of *A. terreus* control was inactivated (function inferred from sequence similarities)
A. terreus lovF (LDKS)	9296 1731	the *lovF* gene of *A. terreus* control was inactivated (function inferred from sequence similarities)
A. nidulans lovB+lovC	9442 1750	plasmid inserted in the chromosome containing the zeocin resistance marker and *lovC* under the control of *alcA* promoter added to *A. nidulans lovB*

2. Incorporation Experiments with Intact Cells for the Elucidation of Lovastatin (1) and Compactin (5) Biosynthesis

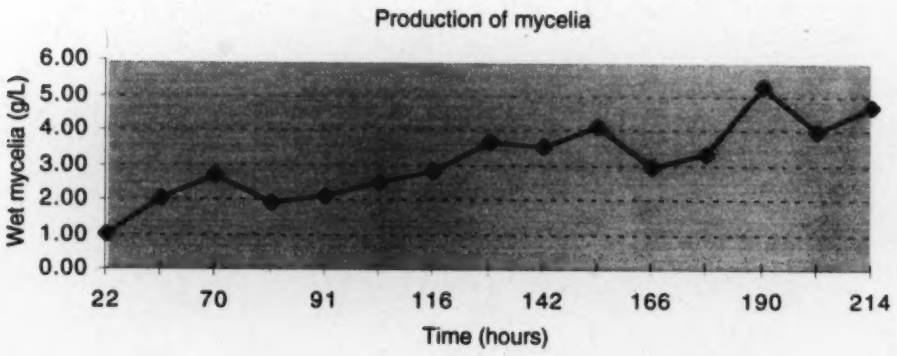
2.1 Synthesis of labelled diketides and incorporation studies into compactin (5) using *Penicillium aurantiogriseum*

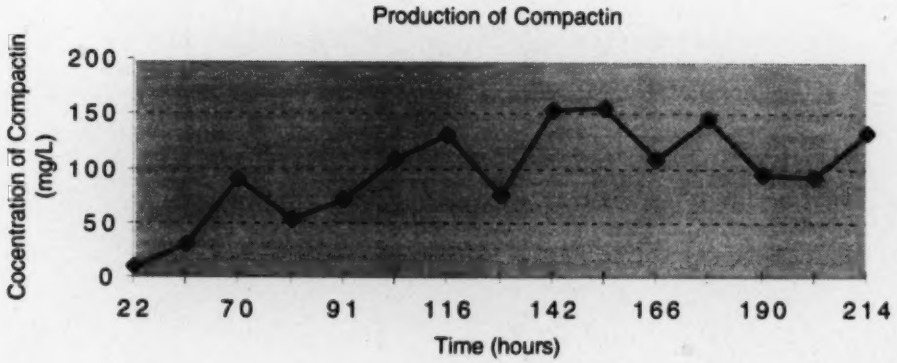
Fermentation of *Penicillium aurantiogriseum* ATCC 60762 produces both compactin (**5**) (100 mg/L of cultures) and ML-236C (**25**) (35 mg/L of cultures). The incorporation of ^{13}C-labeled precursors into polyketides normally proceeds at a low rate (ca. 1.0%), and therefore it is necessary to produce a sufficient amount of metabolite for detection of the labels by ^{13}C NMR. Hence a preliminary compactin production curve and a growth curve of *P. aurantiogriseum* were constructed by quantifying both the wet mycelia and the amount of compactin (**5**) present in the cultures over a nine-day period (Figure 18). The results suggest that feeding of labelled precursors could start on the second day, when the production of compactin is increasing.

Earlier reports show that suitably functionalized PKS chain elongation intermediates, administered as their *N*-acetylcysteamine (NAC) thioesters which may mimic coenzyme A thioesters, can be incorporated intact into various polyketides.[3-7] Thus, appropriately labeled precursors can be used to help establish the exact sequence of events in the chain assembly of secondary metabolites in both bacteria and fungi. In order to test the validity of the proposed pathway to lovastatin (**1**), labeled NAC thioesters of hypothetical precursors (in Scheme 2) could potentially be incorporated. Earlier studies of this type in our group using *Aspergillus terreus* failed because of the rapid degradation of the precursors prior to incorporation into lovastatin (**1**).[46] Since the biosynthesis of

compactin (**5**) closely parallels that of lovastatin (**1**),[42f] it seemed possible that *Penicillium aurantiogriseum* (previously called *P. brevicompactum*) could allow intact incorporation of advanced precursors and provide an alternative, but closely related working model.

Figure 18: Compactin production curve and growth curve of *P. aurantiogriseum*

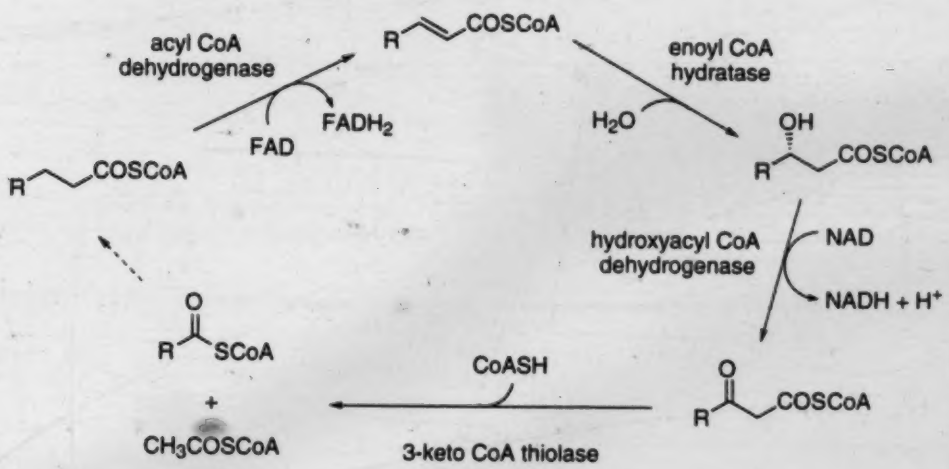




A common cause of the failure to load intact precursors onto PKSs is the competitive degradation of these compounds by highly efficient β- and ω-oxidation enzymes.[95] These ubiquitous pathways for the metabolism of fatty acids lead to small

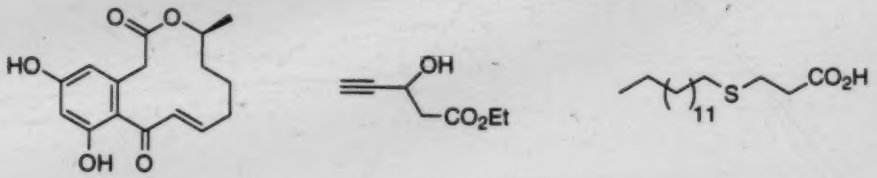
mono- or di-carboxylic acid moieties, thus quickly depleting the pool of fed precursors before intact incorporation can occur. The mechanism of β-oxidation is similar to the reverse of the process of fatty acid formation by FASs (Scheme 7).[95] It involves the α,β-dehydrogenation of the fatty acid CoA ester to the *trans*-enoyl CoA, hydration to the 3-hydroxyacyl CoA, oxidation to the β-ketothioester, and thiolytic cleavage to acetyl CoA and an acyl CoA shortened by two carbons.

Scheme 7: Mechanism of β-oxidation



This process can be retarded by the addition of inhibitors, as utilized in the intact incorporation of di- and tetra-ketides into dehydrocurvularin (**4**).[5] Ethyl-3-hydroxy-4-pentynoate (**26**) and 3-tetradecylthiopropanoic acid (**27**) are two known β-oxidation inhibitors (Figure 19). Compound **26** is probably oxidized to a Michael acceptor, whereas **27** is known to inhibit dehydrogenases.[95]

Figure 19: Dehydrocurvularin (**4**) and β-oxidation inhibitors **26** and **27**



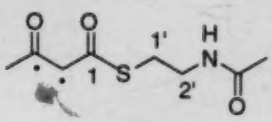
4 **26** **27**

During the ω-oxidation of long chain fatty acids, hydroxylation at the alkyl terminus to ω-hydroxymonocarboxylic acids by cytochrome P450 enzymes is followed by oxidation to the dicarboxylic acids, which are in turn further metabolized by β-oxidation.[96] Since the precursors administered in the present studies were short chains, ω-oxidation was not a concern.

The use of α,β-dilabeled precursors in polyketide incorporation studies allows for the detection of degradation, as *in vivo* cleavage by β-oxidation would generate singly labeled acetates. These are unlikely to recouple to a doubly labeled species due to dilution by unlabeled acetate from the cultures. The intact incorporation of two adjacent carbons with ^{13}C can be detected by ^{13}C NMR because of the low natural abundance of ^{13}C and the large one bond coupling constants observed ($^1J_{CH}$ and $^1J_{CC}$). Confirmation can be provided by isotope ratio using mass spectrometry (MS).

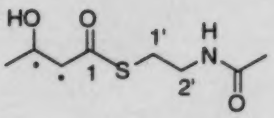
The diketides **28a** and **29a** are NAC ester homologues of the first intermediates in the proposed biosynthesis of both lovastatin (**1**) and compactin (**5**) (Figure 20). Compound **28a** was prepared by Dr. Yaoquan Liu as described in his thesis.[97] Compound **29a** was synthesized and both diketides were fed separately *to P. aurantiogriseum* along with the β-oxidation inhibitor **26**.

Figure 20: Diketides **28**, **28a**, **29**, and **29a**



28 • = ^{12}C

28a • = ^{13}C

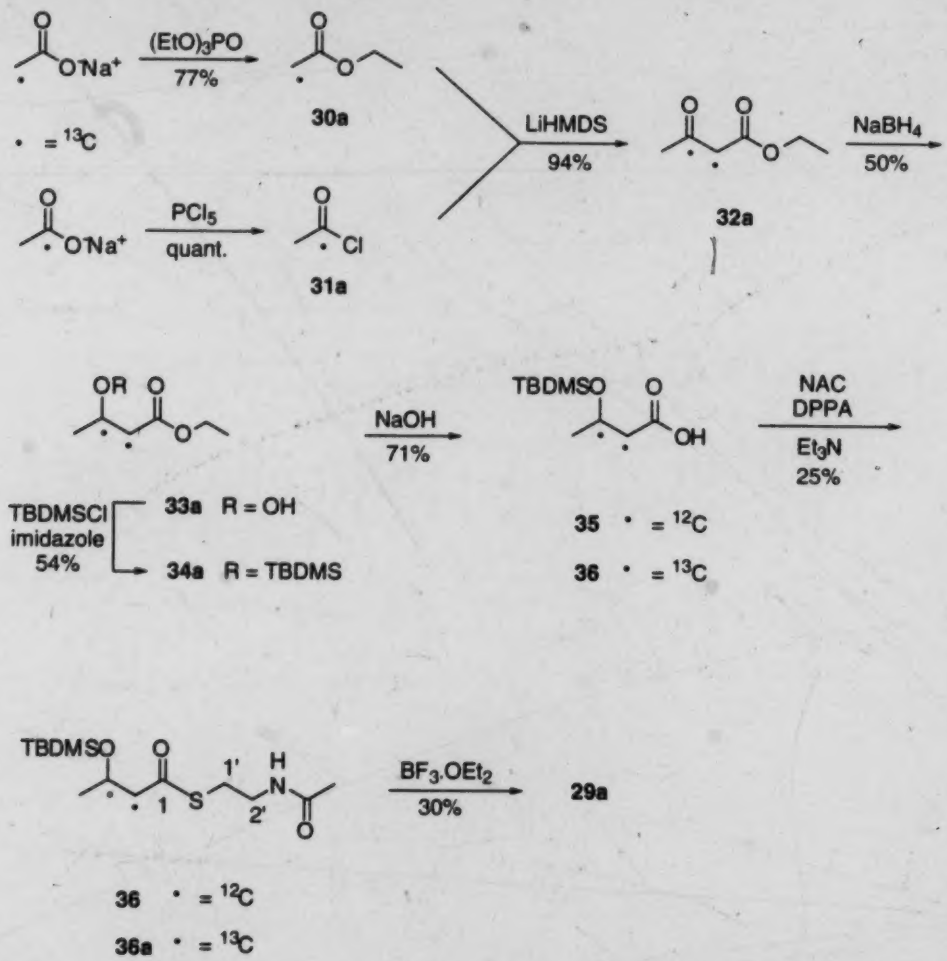


29 • = ^{12}C

29a • = ^{13}C

Synthesis of the diketide 29a. The previously reported synthesis of [2,3-^{13}C]-(3*R*)-hydroxybutyric acid NAC thioester[5] was modified as shown in Scheme 8 to afford **29a**. The synthetic strategy was first optimized by the preparation of unlabeled **29**, although the labeled intermediates will be discussed for simplicity. Labeled sodium acetate is a relatively inexpensive source of ^{13}C. Sodium [2-^{13}C]-acetate reacts with triethyl phosphate to afford [2-^{13}C]-ethyl acetate (**30a**) in 85% yield. The solid-solid reaction of sodium [1-^{13}C]-acetate and phosphorus pentachloride affords pure [1-^{13}C]-acetyl chloride (**31a**) after distillation. Ethyl-[2,3-^{13}C]-acetoacetate (**32a**) results from the condensation of compound **30a** with **31a**. The racemic alcohol **33a** is prepared by reduction of **32a** with sodium borohydride, and is then protected as the *tert*-butyldimethylsilyl ether **34a**. The tetrahydropyranyl- and *tert*-butyldiphenylsilyl-protecting groups could also be used as hydroxyl protecting groups, but whereas the former gives a mixture of diastereoisomers, the latter yields a single isomer of a sterically hindered ester that does not undergo hydrolysis easily. The protected ester **34a** does hydrolyze to the acid **35a** upon treatment with sodium hydroxide.

Scheme 8: Synthesis of diketide **29a**



NAC thioesters are prepared by the coupling of carboxylic acids with NAC. Many reagents have been used for that purpose, including dicyclohexylcarbodiimide (DCC) with catalytic amounts of dimethylaminopyridine (DMAP),[98] carbonyl diimidazole (CDI),[99] and diphenylphosphoryl azide (DPPA).[100] Treatment of the unlabeled acid **35** with NAC in presence of DCC and DMAP affords **36** in 88% yield. Compound **36** is also accessible using DPPA and triethylamine to couple the labeled acid **35** with NAC in quantitative yield. The latter method was chosen for the coupling of acid **35a** with NAC

to give **36a**. Deprotection of the alcohol using boron trifluoride etherate yields the target diketide **29a**.

Incorporation studies of diketides 28a and 29a into compactin (5). The key features for the successful incorporation of advanced precursors into dehydrocurvularin (**4**) are the timing of the precursor addition, the use of β-oxidation inhibitors and high glucose media.[5] High glucose concentrations have been shown to suppress the β-oxidation pathway.[5,101] Therefore the first feeding experiment with *P. aurantiogriseum* was performed after culture transfer into a glucose-rich replacement media and in the presence of inhibitor **26**. The transfer involves filtration of the mycelia after fermentation for two days in production medium (PM) and addition of the collected cells to fresh media. In these feeding experiments, ethanolic solutions of the labeled material **28a** or **29a**, and of the inhibitor **26**, are added simultaneously every 8 hours over a three day period commencing after the transfer into high glucose replacement media. Unfortunately, this procedure leads to a significant decrease in the production of compactin (**5**) (3.3 mg/L). To examine if the decrease of compactin (**5**) production is due to the carbohydrate rich environment or to the transfer itself, the experiment was repeated in the same way, but the filtered mycelia was transferred from the PM cultures into fresh PM instead of replacement media. Once again, the yield of compactin (**5**) was greatly reduced (8.0 mg/L). To ensure that the production of compactin (**5**) is not affected by the β-oxidation inhibitor, the cultures were grown in a similar manner as in the first isolation of compactin (**5**) except that the solution of inhibitor **26** was fed to the cultures starting after two days of growth in PM. The production of compactin (**5**) came back to normal (100 mg/L) despite the inhibitor. Therefore, the final method did not involve the transfer of the mycelia into replacement medium or fresh PM. Rather, the feeding experiments were carried out by direct addition of the labeled compounds to PM cultures starting on the second day of fermentation. None of the conditions used produce any detectable ^{13}C-

^{13}C coupling between C-2 and C-3, nor any other carbons of compactin (**5**) isolated from these feeding experiments. The ^{13}C NMR suggests that scrambling of the labeled atoms occurs, even though traces of intact **29a** could be detected by TLC.

The success of incorporation studies sometimes requires optimization of the conditions, and it is common to undertake many experiments to achieve this. Previous attempts by Dr. Yuko Yoshizawa and Dr. David J. Witter to incorporate a number of labeled intermediates into lovastatin (**1**), either in the presence or absence of various ω- or β-oxidation inhibitors,[4] using different fungal strains, sometimes with the help of protoplasts,[102] saponins,[103] or 2,6-*O*-dimethyl-β-cyclodextrin,[104] were all unsuccessful. Apparently very rapid degradation of the precursors prevents their loading onto the PKS system. A possible cause may be rapid hydrolysis of the NAC esters to the parent acids, which are known not to be loaded onto PKS enzymes.[105] Further investigations on the biosynthesis of lovastatin (**1**) therefore employed genetically engineered fungal strains.

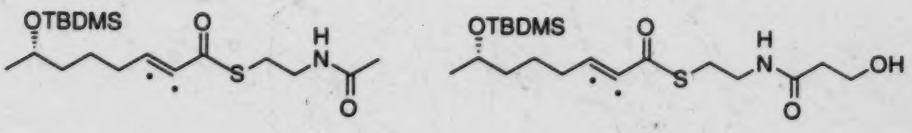
2.2 Studies with *A. terreus lovE* and *A. terreus* XS-1P strains

Lovastatin production. Studies by Professor C. R. Hutchinson and coworkers show that the *lovE* gene contains a $Zn(II)_2Cys_6$ binuclear cluster indicating that it might be a regulatory gene.[94] Both the *A. terreus lovE* and XS-1P strains resulted from transformation of the wild type strain with the *lovE* gene under the control of different promoters. As expected, they are lovastatin overproducers. *A. terreus lovE* produces approximately five times more lovastatin (**1**) than the *A. terreus* control, while *A. terreus* XS-1P yields 30 times more lovastatin (**1**) than the control strain. No new metabolites are observed for either strain under the purification conditions used.

Incorporation studies using protoplasts. The use of protoplasts in incorporation studies rely on increasing the permeability of the fungal cell membrane to

ease the precursor's transport into the cell. Cyclodextrin derivatives are believed to stimulate the uptake of precursors, although a detailed mechanism for this has not been suggested.[104] Dr. Yaoquan Liu has examined an active transport strategy to enhance the already successful incorporations of di- and tetraketides into dehydrocurvularin (**4**) from *Alternaria cinerariae*.[97] The acetyl end of *N*-acetylcysteamine was functionalized to allow an easily cleavable linkage to be formed between the precursor and a carrier. The carrier which may be a peptide or a carbohydrate, could allow transport of the precursor accross the cell membrane. Within the cell the linkage could be broken by hydrolytic enzymes, leaving the advanced intermediate available for incorporation. As a preliminary test of this idea, the tetraketides **37** and **38** were prepared by Dr. Yaoquan Liu, however, intact incorporation could be achieved only with the NAC thioester **37** (Figure 21).

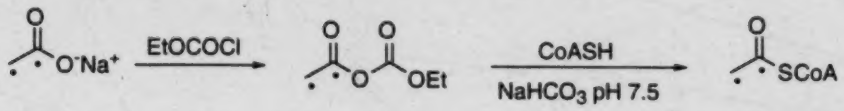
Figure 21: Tetraketides **37** and **38** for incorporation studies with dehydrocurvularin (**4**)



• = ^{13}C **37** **38**

The incorporation of [1,2-$^{13}C_2$]-acetyl CoA (**39a**) into lovastatin (**1**) was investigated with protoplasts from *A. terreus* XS-1P. Compound **39a** was chosen for its large size and easy accessibility (Scheme 9). The reaction of sodium [1,2-$^{13}C_2$]-acetate with ethyl chloroformate in THF was followed by IR until the transformation to the mixed anhydride **40a** was complete. The crude anhydride **40a** was added to an aqueous solution of coenzyme A at pH 7.5. Formation of the target thioester **39a** was monitored with the sodium nitroprusside test for thiols.

Scheme 9: Synthesis of [1,2-$^{13}C_2$]-acetyl CoA (**39a**)



* = ^{13}C **40a** **39a**

With compound **39a** available, protoplasts of *A. terreus* XS-1P were prepared using three different procedures along with two controls.[102b,c] The washed mycelia collected by filtration was incubated with commercially available lysing enzymes from *Trichoderma harzianum* in the presence of either of the osmotic stabilizers magnesium sulfate, potassium chloride, or mannitol. The 'protoplasts' thus generated were gently collected, washed and transfered into fermentation media. Feeding of an aqueous solution of compound **39a** was immediately begun, and continued every 8 hours for two days. Although the cells treated with lysing enzymes looked very different from the control cells, no intact incorporation into lovastatin (**1**) was observed. The production of lovastatin (**1**) did not seem affected by the protocol for protoplast preparation.

Many factors could explain the absence of ^{13}C incorporation. The lysing enzymes may not have fully degraded the fungal cell walls, or if they did, the regeneration of these could have been fast enough to prevent the occurrence of enough incorporation. Alternatively, intact cell membranes could still prevent access of the anionic charged CoA moiety to the PKS enzyme.

2.3 Production of novel metabolites by *A. nidulans lovB*

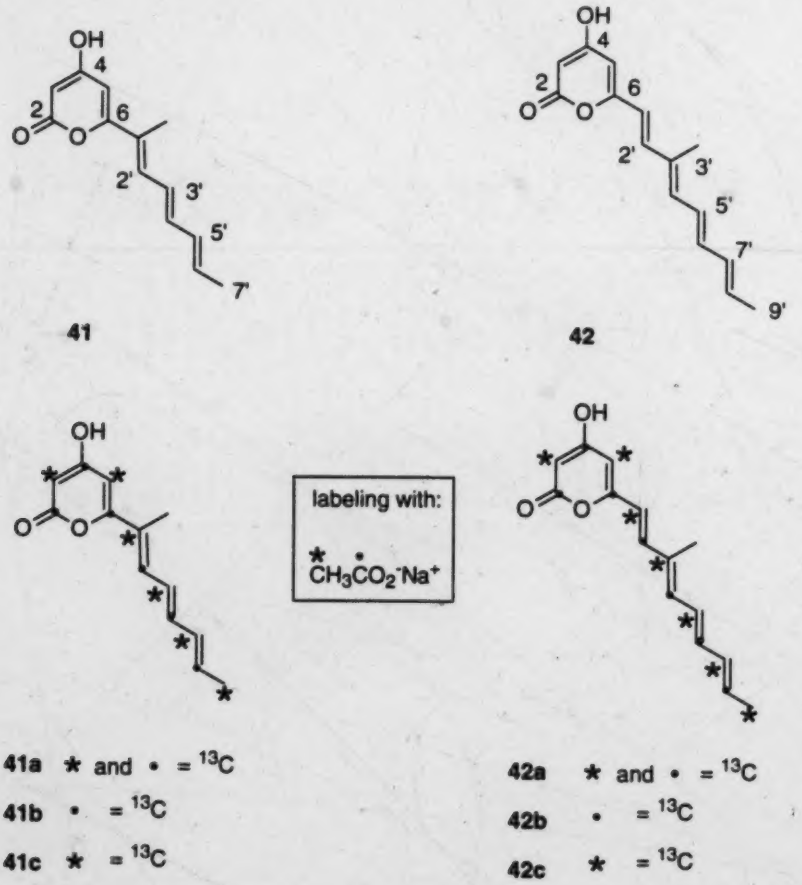
Isolation and structure elucidation of novel metabolites 41 and 42 resulting from the cloning of the *lovB* gene from *A. terreus* into the heterologous host *A. nidulans*. From sequence similarities, it was believed that the open reading frame 4 (ORF 4), later called *lovB*, encoded the iterative type I LNKS (see

Table 1). As mentioned above, the gene includes the domains characteristic of PKSs (KS, AT, DH, ER, ACP), plus a methyl transferase (MeT) domain, and a *C*-terminal condensation domain, which could be involved in the cyclization of the polyketide. To further investigate the role of *lovB*, the Hutchinson group cloned and overexpressed the gene into the heterologous host *A. nidulans* under the control of the *alcA* promoter.[94] Since 4a,5-dihydromonacolin L (**11**) was believed to be the first released product of the putative LNKS, *A. nidulans lovB* was expected to produce this compound. Our fermentation of *A. nidulans lovB* strain under inducing conditions, produced yellow cultures in contrast with the clear-colored *A. nidulans* wild type cultures. Sterigmatocystin, a secondary metabolite naturally produced by the wild type strain could not be detected in *A. nidulans lovB*. Its production was restored after repression of the *alcA* promoter, therefore suggesting a shortage of acetate in the host fungi.

The yellow pigments were extracted in ethyl acetate after acidification of the cultures and purified by HPLC to afford 4-hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (**41**) as a yellow powder, and 4-hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (**42**) as an orange powder (Figure 22). As an alternative purification method, the addition of XAD-2 resin to the filtered cultures was attempted, but the target compounds **41** and **42** were not absorbed on the resin. Crystallization of these compounds did not provide pure crystals for X-ray analysis because of competing decomposition.

The ^{1}H and ^{13}C NMR assignments of **41** and **42** were assisted by 2D NMR (GCOSY, HMQC, HMBC, see Figure 23) and by biosynthetic labeling with ^{13}C-acetates. The feeding of sodium acetate (total of ca. 3 g/L) to the *A. nidulans lovB* cultures increases the production of both **41** and **42**, probably due to a lack of acetate in the host. The metabolism of acetate in the wild type *A. nidulans* does not normally include the biosynthesis of these yellow pigments, hence the shortage. However, the administration of too much sodium acetate (total of ca. 5g/L), inhibits the production of **41** and **42**.

Figure 22: The yellow pigments unlabeled: **41** and **42**; and labeled: **41a**, **41b**, **41c**, **42a**, **42b**, and **42c**



41

42

41a * and • = ^{13}C

41b • = ^{13}C

41c * = ^{13}C

42a * and • = ^{13}C

42b • = ^{13}C

42c * = ^{13}C

The instability of **41** and **42**, the extended conjugation system showing many long range correlations, and the proximity of many quaternary carbons, made the elucidation of the carbon skeleton difficult without ^{13}C COSY. Surprisingly, the labeling by mono- and di-^{13}C- sodium acetate is as high as 70% per carbon based on NMR and

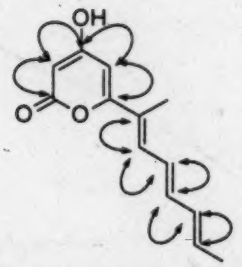
MS for compounds **41a, 41b, 41c, 42a, 42b,** and **42c** (Figure 22). No incorporation of ^{13}C is observed for the 1'-Me of **41** and for the 3'-Me of **42**, suggesting an *S*-adenosyl-*L*-methionine origin for these carbons, as was shown for lovastatin.[42a]

The high incorporation rate allows efficient APT, DEPT and ^{13}C COSY experiments on small quantities. APT and DEPT techniques help resolve the overlapping carbon signals and determine the biosynthetic origin of each carbon atom. The acetate units are arranged in a linear head-to-tail fashion, as predicted for the six acetate units of **41**, and the seven units of **42** (Figure 23). The ^{13}C COSY spectrum confirms the proposed carbon skeleton through the observed correlations between adjacent carbons, including the quaternary atoms.

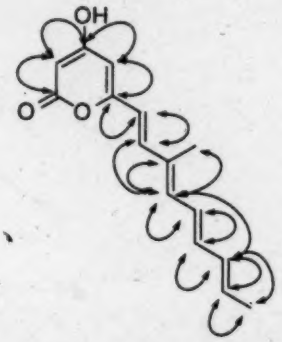
NOE experiments further delineate the molecular structures (Figure 23). The stereochemistry of the double bond at C-1' ($\Delta_{1'}$) of both **41** and **42** could not be assigned from the coupling constants. NOE experiments involving irradiation of 1'-Me of **41** resulted in signal enhancement factors of about 1% for H-5 and H-3'. Irradiation of 3'-Me of **42** also gave 1% signal enhancement for H-1' and H-5'. This establishes the (*E*) stereochemistry for both. NOE also confirmed the (*E*) stereochemistry of $\Delta_{1'}$, $\Delta_{3'}$, for **41** and **42** and $\Delta_{5'}$ for **42**, already assigned from the coupling constants.

Finally, the hydrogenation of compounds **41** and **42** with a catalytic amount of palladium on charcoal under a hydrogen atmosphere affords 4-hydroxy-6-(1-methylhept-1-yl)-2-pyrone (**43**) and 4-hydroxy-6-(3-methyl-non-1-yl)-2-pyrone (**44**) respectively (Scheme 10). Labeled compounds **43a** and **44a** were prepared from ^{13}C-labeled **41a** and **42a** to allow ^{13}C COSY experiments on small amounts. Although more stable than their precursors, **43** and **44** also decomposed over time.

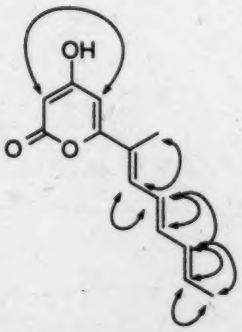
Figure 23: Results of selected 2D NMR experiments on compounds **41** and **42**



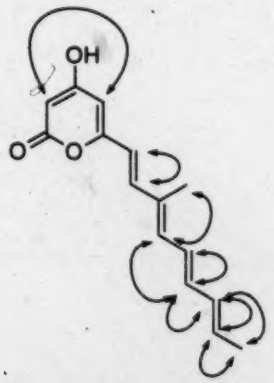
^{13}C COSY correlations for **41a**



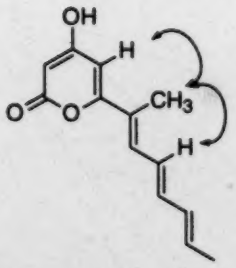
^{13}C COSY correlations for **42a**



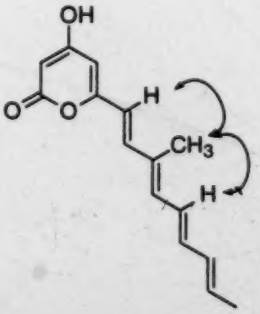
^{1}H COSY correlations for **41**



^{1}H COSY correlations for **42**

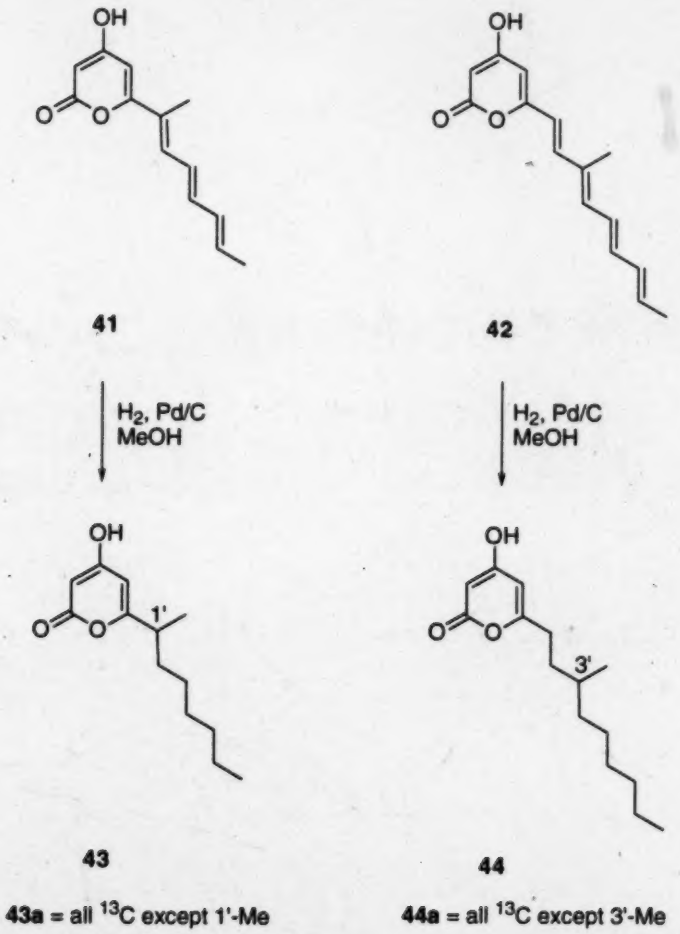


NOE correlations for **41**



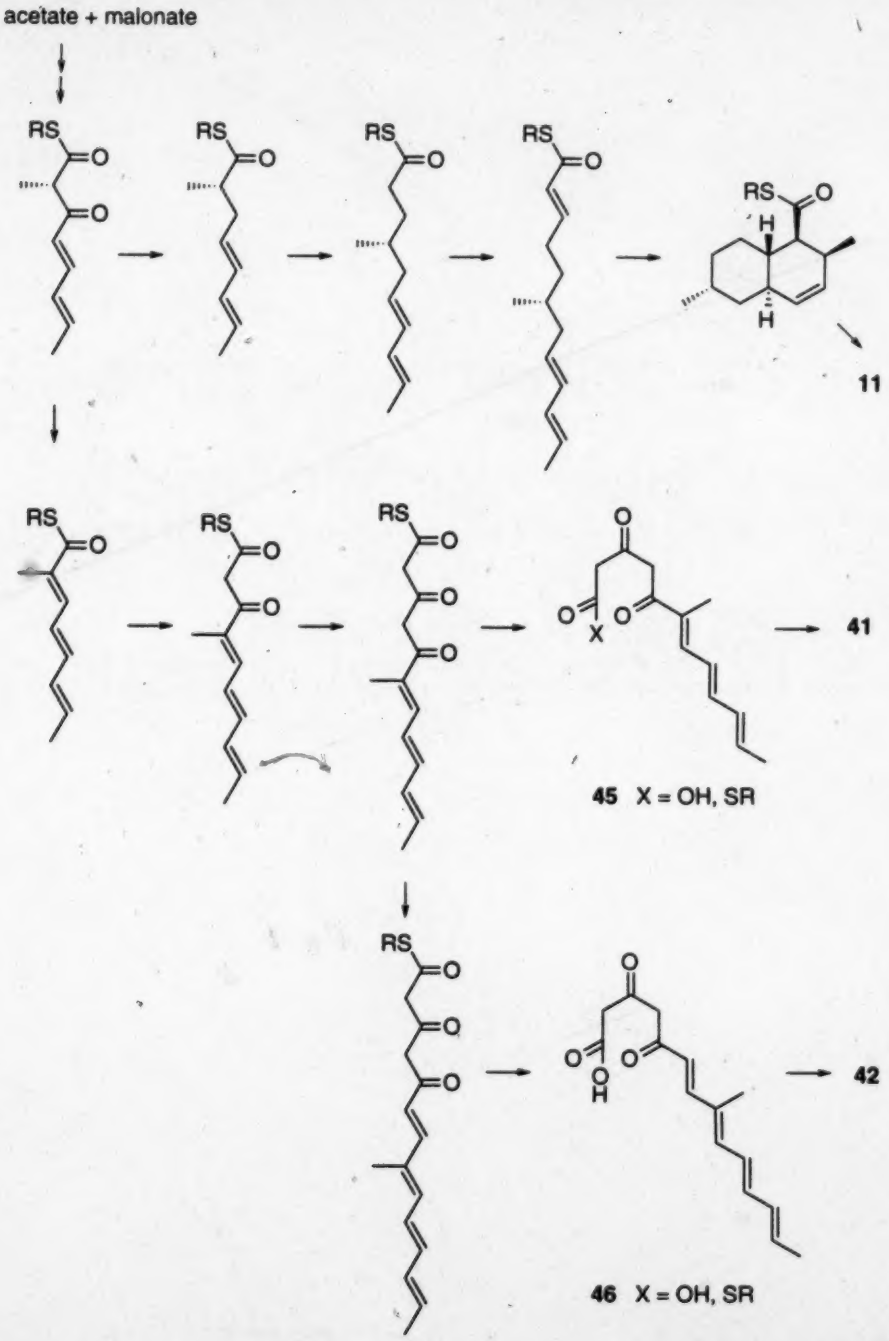
NOE correlations for **42**

Scheme 10: Hydrogenation of compounds **41** and **42**



The production of **41** and **42** by *A. nidulans lovB* can be rationalized as follows. If it is assumed that **45** and **46** are the molecules released by the LNKS enzyme (Scheme 11), then spontaneous ring closure would afford **41** and **42**. Alternatively, the failure of "correct" reduction could lead to direct cyclization of the PKS-bound thioester intermediate. The products **41** and **42** obtained show critical similarities with the expected polyketide intermediates on the pathway towards **11**.

Scheme 11: Proposed biosynthesis of compounds **41** and **42**



The head-to-tail arrangement of the acetate units confirms that **41** and **42** are polyketides made by LNKS in the modified *A. nidulans*. The presence of a methyl group potentially derived from SAM at the seventh carbon from the methyl terminus of **41** and **42** demonstrates that the MeT is still active and regioselective (methylation at the same position as on the lovastatin nonaketide chain). The introduction of the first three acetate units and their reduction to two double bonds correlates to normal LNKS function. Problems arise when the fourth acetate unit is introduced. After the methyl group of SAM was added by the MeT domain, the ketone formed should be reduced completely to a methylene unit but the reduction is stopped at the olefinic stage. This suggests that the ER domain of LNKS does not function. Two or three more acetate units are nevertheless added but then the chain elongation is stopped possibly due to recognition problems, or spontaneous cyclization. Sequence analysis of the ER domain of LNKS suggests that it may be inactivated,[94] and this is now confirmed by the failure of LNKS to reduce the Δ_1 double bond present in **41** and **42**. The production of **41** and **42** can be explained by the potential requirement of a second protein for LNKS to produce 4a,5-dihydromonacolin L (**11**) (demonstrated below). The second protein might act as an enoyl reductase, or as an activator of LNKS or of its ER.

2.4 Production of metabolite 41 by *A. terreus lovC*

Many mutants of *A. terreus* were prepared by the Hutchinson group in which one ORF was selectively disrupted.[94] When the ORF 6, later called *lovC*, is disrupted our fermentation of *A. terreus lovC* results once again in the production of yellow metabolites. Extraction and isolation in the same manner as for *A. nidulans lovB* affords **41** (confirmed by IR, UV, ^{1}H NMR and ESMS). It appears that **42** is produced as well (from the HPLC trace) although in amounts too small for full characterization. The extra

gene in *A. nidulans lovB* is overexpressed, but this is not the case in the *A. terreus lovC* mutant, hence the smaller quantities obtained in these experiments.

The production of compound **41** by both the *A. nidulans lovB* and the *A. terreus lovC* mutant strains suggests that at least both *lovB* and *lovC* are required for the production of 4a,5-dihydromonacolin L (**11**). Sequence analysis shows that *lovC* encodes a protein that has high similarities to an ER domain. Since LNKS ER may be inactivated, LNKS and the LovC protein might associate to achieve the biosynthesis of 4a,5-dihydromonacolin L (**11**).

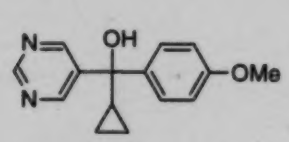
2.5 *A. terreus lovA* does not produce 4a,5-dihydromonacolin L (11)

A 4a,5-dihydromonacolin L producing strain is very desirable since it would allow biosynthetic studies to verify the involvement of a PKS-catalyzed Diels-Alder reaction. For a mutant strain to be useful in verifying the potential Diels-Alderase activity, the polyketide chain has to be assembled at least to the hexaketide stage (see Scheme 2). It was believed that the first polyketide released by the lovastatin PKS was the nonaketide dihydromonacolin L (**11**). The elucidation of the post-PKS steps by Endo and coworkers[44] suggests that probably two cytochrome P450 dependant enzymes may be involved in this process. Sequence analysis indicated that the ORF 3 (*lovA* or P450I) and the ORF 17 (P450II) might encode cytochrome P450 dependant enzymes. Therefore, the disruption of either could potentially lead to the production of **11** by blocking the post-PKS steps. The *A. terreus lovA* mutant was prepared by the research group of Professor Hutchinson and upon analysis of the cultures, no 4a,5-dihydromonacolin L (**11**) could be detected. Mr. John Sorensen in our group is presently working on the structure elucidation of new metabolites produced by this strain.

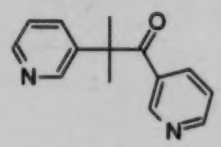
2.6 Inhibition of *A. terreus* XS-1P with ancymidol

Cytochrome P450 dependant oxidations[106] are common tailoring processes in the assembly of secondary metabolites. These transformations usually take place in the late stages of the biosynthesis and can be important for biological activity. Various inhibitors of these enzymes have been developed as clinical drugs and plant growth regulators,[107] including ancymidol (**47**), metyrapone (**48**), and S-3307D (**49**) (Figure 24). Their heteroaromatic moieties reversibly bind the lipophilic domain and the heme iron of P450 dependant enzymes, thereby blocking recognition of the substrate and molecular oxygen. Based on this mechanism, P450 inhibitors have been successfully used to suppress the oxidative modification of several natural products, resulting in the accumulation of less oxidized intermediates. Ancymidol and metyrapone were effectively used by Oikawa *et al.* for biosynthetic studies of betaenone B[108] and chaetoblosin[109] produced by *Phoma betae* and *Chaetomium subaffine*, respectively.

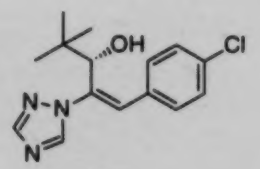
Figure 24: Cytochrome P450 dependant enzyme inhibitors



47



48



49

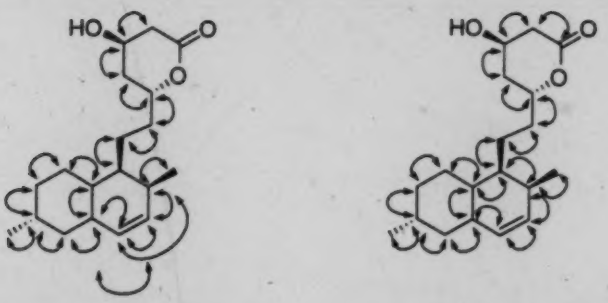
In an effort to produce the less oxidized derivative of lovastatin (**1**), 4a,5-dihydromonacolin L (**11**), ancymidol was added to *A. terreus* XS-1P cultures every eight hours for five days. The production of new metabolites and of lovastatin (**1**) was monitored by HPLC. The ancymidol inhibited *A. terreus* XS-1P cultures were compared against normal *A. terreus* XS-1P cultures. The cultures treated with ancymidol (**47**) show production of monacolin J (**14**). The lovastatin (**1**) production is reduced by a

factor of three, whereas the wet mycelia production is down to 70% compared to the non-inhibited *A. terreus* XS-1P cultures.

2.7 Production of 4a,5-dihydromonacolin L (11) by *A. nidulans lovB+lovC*

The production of the shunt compounds **41** and **42** by both *A. nidulans lovB* and *A. terreus lovC* suggests that these two genes are required for the PKS transformations. To confirm the hypothesis that the two proteins encoded by the *lovB* and the *lovC* genes may interact to produce 4a,5-dihydromonacolin L (**11**), *lovC* under the control of the *alcA* promoter, was cloned and overexpressed in *A. nidulans lovB* by our collaborators at the University of Wisconsin.[94] The resulting recombinant strain, *A. nidulans lovB+lovC*, when fermented under *alcA* inducing conditions, produces yellowish cultures resembling *A. nidulans lovB*. Analysis show that the pigmentation is probably caused by formation of compounds **41** and **42**. However, 4a,5-dihydromonacolin L (**11**) is generated as the major new metabolite of *A. nidulans lovB+lovC*. The production of **41** and **42** may be due to a shortage of NADPH, an excess of the LovB protein relative to Lov*C*, or requirement of a third protein (e.g. ORF5) for full activity. The structure elucidation of **11** employed 2D NMR (GCOSY, HMQC, HMBC, see Figure 25) and biosynthetic labeling with ^{13}C acetate. The labeling by sodium [1,2-$^{13}C_2$]-acetate is again very high: ca. 20% per carbon from NMR and MS. No incorporation of ^{13}C is observed for the 6-Me of **11**, indicating the *S*-adenosyl-*L*-methionine origin of this carbon, as was shown for lovastatin (**1**).[42a] The high incorporation rate allows efficient ^{13}C COSY experiments on small quantities to confirm the carbon skeleton, resolves overlapping carbon signals, and confirms the polyketide origin of **11**. The ^{1}H and ^{13}C NMR assignment of compound **11** deduced from these numerous experiments shows discrepancies from what was originally reported by Endo and coworkers.[44d]

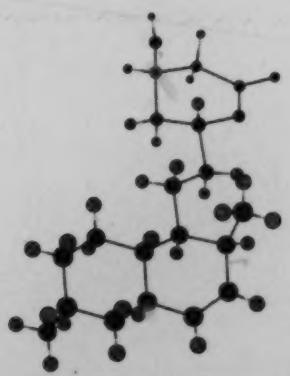
Figure 25: Results of selected 2D NMR experiments on compounds **11** and **11a**



^{1}H COSY correlations for **11** ^{13}C COSY correlations for **11a**

The structure of compound **11** was confirmed by X-ray crystallography (Figure 26).[110] The X-ray crystallographic analysis provides only the relative stereochemistry of compound **11**; the absolute stereochemistry is inferred from the known configuration of lovastatin (**1**) and compactin (**5**).[31] 4a,5-Dihydromonacolin L (**11**) has been shown by Endo and coworkers to be an intermediate in the lovastatin biosynthetic pathway, and this was also confirmed by us as described below (section 3.5).[44]

Figure 26: X-ray crystal structure of compound **11**



In light of the increased production of the pigments **41** and **42** following sodium acetate feeding, the effects of the administration of sodium acetate or diethyl malonate on the production of 4a,5-dihydromonacolin L (**11**) by *A. nidulans lovB+lovC* were investigated. The same pulse feeding technique as used before was adopted. Thus, in

two separate experiments, an aqueous solution of sodium acetate or neat diethyl malonate was administered to *A. nidulans lovB+lovC* cultures every eight hours for two days commencing after the transfer into AMM production media. The addition of sodium acetate did not affect the production of **11**, but surprisingly, diethyl malonate decreased the yield of **11** below the detection limit. Addition of a large excess (more than 5 g/L of cultures) of sodium acetate gives a decrease in the production of **41** and **42** by *A. nidulans lovB* (see above). In principle, diethyl malonate should be quickly hydrolyzed to malonic acid *in vivo* and transformed into the CoA thioester for use in polyketide biosynthesis. It is possible that the monoethyl malonate or diethyl malonate may inhibit the PKS, or repress its formation.

2.8 *In vivo* incorporation studies of the diketide 29a into 4a,5-dihydromonacolin L (11) using *A. nidulans lovB+lovC*

The rapid degradation of the labeled precursors administered to *A. terreus* and *P. aurantiogriseum* might be less prominent in another fungal strain like *A. nidulans*. *In vivo* studies with advanced labeled intermediates on the pathway to **11** could confirm the identity of enzyme-bound species on the biosynthetic pathway. Hence, an ethanolic solution of the diketide **29a**, prepared as described above, and a solution of the β-oxidation inhibitor **26**, were pulse fed to cultures of *A. nidulans lovB+lovC* every four hours for three days commencing after transfer into AMM production media. The 4a,5-dihydromonacolin L (**11**) isolated after this experiment does not show any ^{13}C-^{13}C coupling for the ^{13}C NMR signals of C-2 and C-3, nor for any other carbons. Apparently the precursor degradation is still more rapid than utilization by the PKS, as was also seen for *A. terreus*.

2.9 Significance

The biosynthesis of 4a,5-dihydromonacolin L (**11**) requires the combined activities of only LovB and LovC proteins. How these two enzymes interact remains unknown. The LovB protein alone catalyzes the assembly of the yellow pigments **41** and **42**. The presence of a SAM-derived methyl group at C-1' of both **41** and **42** indicates that methylation by the LovB protein occurs at the β-diketo stage, before reduction to the double bond which would be hydrogenated by *lovC*. Conversely, the incorporation of ^{2}H at C-2' of lovastatin (**1**) reported before[42] is consistent with methylation of the side chain diketide after enoyl reduction by LDKS. Thus it appears that two methylation pathways maybe used by fungi.

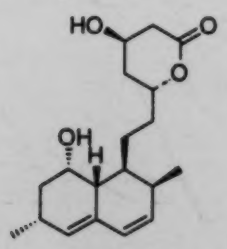
Sequence comparisons suggest that the *lovB* gene encodes a PKS that may have an inactive ER domain and may lack the NADPH binding site. Although *lovC* encodes an apparent enoyl reductase sequence, its NADPH binding site does not follow the common consensus sequence either. This suggests that in the interaction of LovB and LovC proteins, LNKS assembles the acetate units, transforms the appropriate ketones to hydroxyl groups, and selectively dehydrates some of them. The LovC protein probably accomplishes the three enoyl reductions required for the biosynthesis of 4a,5-dihydromonacolin L (**11**). Alternatively, the required activity may result through the complexation of the LovC protein and LNKS (*lovB*). Numerous unsuccessful attempts to incorporate advanced precursors (e.g. diketides) into **11** with both *A. terreus* and *A. nidulans* suggest that fully or partially purified PKS enzymes will be required for this.

3. Studies on Post-PKS Transformations leading to Lovastatin

3.1 Production of monacolin J (14) by both the *A. terreus lovD* and the *A. terreus lovF* mutants

In the last step of the biosynthesis of lovastatin (**1**), the oxidized nonaketide monacolin J (**14**), and the diketide (2*R*)-methylbutyric acid (**15**) are condensed through esterification. In the lovastatin gene cluster, *lovD* was attributed an esterase function and *lovF* a PKS function by sequence comparisons. LDKS (*lovF*) is the enzyme believed to be responsible for the biosynthesis of **15**. To confirm this assignment, strains *A. terreus* with *lovD* and *lovF* genes disrupted were prepared by Professor Hutchinson's research group.[94] If the LovD protein is responsible for the esterification of monacolin J (**14**) with (2*R*)-methylbutyric acid (**15**) *A. terreus lovD* should produce both **14** and **15** but no lovastatin (**1**). If the *lovF* gene encodes for LDKS then *A. terreus lovF* should produce monacolin J (**14**) but fail to link it to (2*R*)-methylbutyric acid (**15**) because of its absence. Upon analysis of the cultures of these two strains, no production of lovastatin (**1**) was found, and the only new metabolite observed was monacolin J (**14**). This confirms the esterase and LDKS functions proposed for the LovD and LovF proteins, respectively.

Figure 27: Monacolin J (**14**)

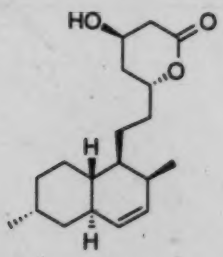


14

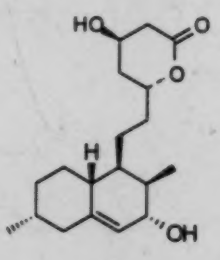
3.2 Investigation of the production of the side chain diketides 15, 50, and 51 in the *A. terreus lov C* mutant

The *A. terreus lovC* mutant does not produce lovastatin (**1**) nor any of the post-PKS intermediates **11**, **12**, **13** and **14** (Figure 27 and 28). Nevertheless, this strain should still contain all the activities required to assemble lovastatin (**1**), except for the transformations from **45** and/or **46** to 4a,5-dihydromonacolin L (**11**). Therefore the *A.*

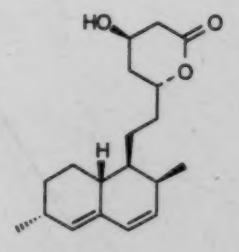
Figure 28: Compounds **11**, **12**, **13**, **45**, and **46**



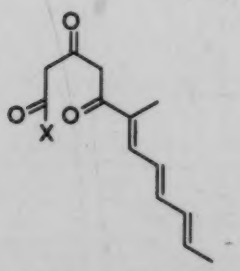
11



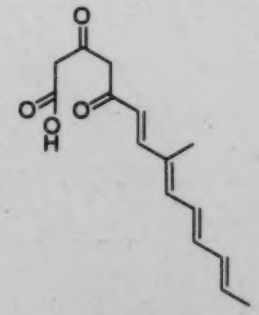
12



13



45 X = OH, SR

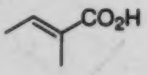


46 X = OH, SR

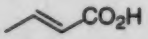
terreus lovC mutant represents an ideal strain to verify the post-PKS biosynthetic reactions leading to lovastatin (**1**). LDKS (encoded by *lovF*) should still be active and the diketide **15** should be produced by *A. terreus lovC* unless the enoyl reductase function of LovC is required for full LDKS activity. If this were the case, tiglic acid (**50**) or crotonic

acid (**51**) (Figure 29) would be produced instead. Extensive analysis of the *A. terreus lovC* cultures did not reveal the presence of any of the three possible diketides (i.e. **15**, **50**, **51**). This suggests either that LDKS requires the activity of *lovC* to release the diketide **15**, or that it might be released directly to the esterase only in the presence of monacolin J (**14**) (see below).

Figure 29: Tiglic acid (**50**) and crotonic acid (**51**)



50



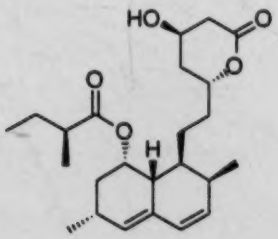
51

3.3 Investigation of the incorporation of monacolin J (14) into lovastatin (1) using the *A. terreus lovC* mutant

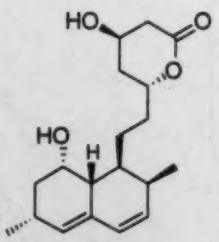
As shown above, no (2*R*)-methylbutyric acid (**15**), tiglic acid (**50**), nor crotonic acid (**51**) were detected in the *A. terreus lovC* mutant cultures, suggesting that the diketide formed might remain loaded as a thioester on the enzyme (LovF protein or LDKS) until monacolin J (**14**) is available to the esterase (LovD protein). At that point, both monacolin J (**14**) and the diketide **15** may be transferred to the esterase to be transformed to lovastatin (**1**). As a preliminary test of this hypothesis, monacolin J (**14**), prepared by hydrolysis of lovastatin (**1**) by Dr. Joanna Harris (Figure 30), was administered to the *A. terreus lovC* mutant every six hours for two days starting half a day after the mycelia was transferred into AMM production media. The yield of lovastatin (**1**) observed accounted for as much as 70% of the monacolin J (**14**) injected. This experiment proves that unlike the LovB protein, LDKS (*lovF*) does not require the LovC protein. This also supports the proposal that all of the LDKS proposed domains,

including the enoyl reductase, are active,[94] and that the diketide **15** is not released until it is esterified with monacolin J (**14**).

Figure 30: Lovastatin (**1**) and monacolin J (**14**)



1

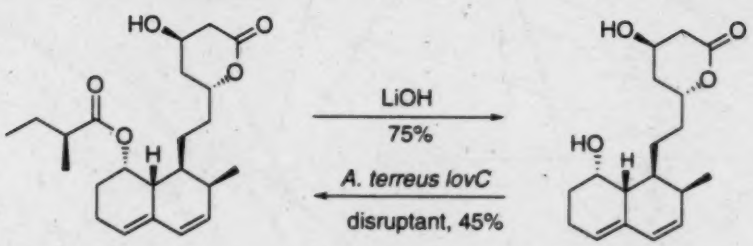


14

3.4 Investigation of the incorporation of 6-desmethylmonacolin J (52) into compactin (5) using the *A. terreus lovC* mutant

In order to examine whether the LovD protein (esterase) of *A. terreus* absolutely requires monacolin J (**14**), 6-desmethylmonacolin J (**52**) was added to the *A. terreus lovC* mutant cultures to determine whether compactin (**5**) would be produced instead of lovastatin (**1**). 6-Desmethylmonacolin J (**52**) is readily prepared in 75% yield by the hydrolysis of compactin (**5**) with lithium hydroxide (Scheme 12). Compound **52** was pulse fed to the *A. terreus lovC* mutant in the same fashion as for the incorporation of monacolin J (**14**). Compactin (**5**) was isolated from the cultures in 45% yield from 6-desmethylmonacolin J (**52**). This demonstrates that the esterase (LovD protein) does not require the methyl group at C-6 for activity.

Scheme 12: Compactin (**5**) and 6-desmethylmonacolin J (**52**)



5 **52**

3.5 *In vivo* incorporation of 4a,5-dihydromonacolin L (11) into lovastatin (1) using the *A. terreus lovC* mutant

In order to confirm that compound **11**, isolated from *A. nidulans lovB+lovC*, is a biosynthetic precursor to lovastatin (**1**) the *A. terreus lovC* mutant strain was used. This strain produces the pigments **41** and **42**, but forms no lovastatin (**1**) even though all the necessary machinery is present except for the enoyl reductase. Thus, 4a,5-dihydromonacolin L (**11**) was added to cultures of the *A. terreus lovC* mutant. Lovastatin (**1**) could be isolated from the cultures in 40% yield from 4a,5-dihydromonacolin L (**11**). This experiment confirms that **11** is an intermediate on the biosynthetic pathway to compound **1**.

3.6 Attempted incorporation of ML-236C (25) into compactin (5) using the *A. terreus lovC* mutant

In order to further investigate the specificity of the post-PKS enzymes which normally transform 4a,5-dihydromonacolin L (**11**) to lovastatin (**1**), ML-236C (**25**) (Figure 31) was tested as a substrate in the *A. terreus lovC* mutant. This compound is the

6-desmethyl analog of monacolin L (**13**). Presumably all biosynthetic enzymes required to oxidize C-8 and add the side chain are present, and the previous experiment showed that the latter process proceeds on the 6-desmethyl system. However, no compactin (**5**) nor ML-236C (**25**) could be detected in the cultures 48 hours after the feeding of **25**. This suggests either that the hydroxylation at C-8 is very specific and requires a methyl at C-6 or that monacolin L (**13**) is not a precursor to lovastatin (**1**) as suggested by Merk researchers.[45] The depletion of compound **25** in *A. terreus lovC* could be due to its metabolism by other enzymes present in the cells.

Figure 31: ML-236C (**25**) is an intermediate in the pathway towards compactin (**5**)

25 **5**

3.7 Significance

Our incorporation studies help clarify the biosynthetic steps involved in the transformation of 4a,5-dihydromonacolin L (**11**) to lovastatin (**1**). LDKS, encoded by *lovF*, constructs (2*R*)-methylbutyric acid (**15**) which remains loaded on the enzyme until monacolin J (**14**) is available. The lack of a TE domain is consistent with the transfer to a separate enzyme. LDKS appears to act like a non-iterative PKS and uses each active site only once to produce the diketide **15**. Whether LDKS is really a modular enzyme, or an

iterative PKS from which the substrate is extracted by the esterase before it can be further processed remains uncertain.

The LovB+LovC protein system manufactures 4a,5-dihydromonacolin L (**11**). This compound is converted to monacolin J (**14**) by post-PKS enzymes, at least one of which, the enzyme carrying out the C-8 hydroxylation, appears to require methylation at C-6. The esterase then combines the diketide **15** with the nonaketide **14** to finally release lovastatin (**1**). This last enzyme shows some substrate flexibility since it transforms a C-6 desmethyl analogue. The substrate specificity of the enzymes could be further tested using the *A. terreus lovC* mutant. For example, incorporation studies with the hexaketide, heptaketide or octaketide NAC esters into lovastatin (**1**) could potentially determine whether or not the LovC protein is required only at the tetraketide stage (when methylation occurs). The specificity of the esterase (LovD protein) for the diketide could be tested by feeding different di-, tri-, or tetraketides to the *A. terreus lovF* mutant. The use of such mutants has the advantage that many experiments can be performed without the use of expensive labels.

Unfortunately, none of the mutant strains presently available allow intact incorporation of advanced intermediates to elucidate the PKS transformations during the biosynthesis of lovastatin (**1**). Hence, the preparation of cell free extracts and enzyme purification are attractive alternatives to achieve this goal.

4. Cell Free Experiments for the Study of Lovastatin Nonaketide Assembly by PKS Enzymes

4.1 Cell free extracts (CFE) of *A. terreus lovE* and *A. terreus* XS-1P

Modifications of the method of Watanabe and Townsend for the preparation of cell free extracts (CFEs) of *Aspergillus parasiticus*[111] were unsuccessfully attempted on lovastatin overproducers *A. terreus lovE* and XS-1P. Variations were examined for methods to break the cells (liquid nitrogen or 4 °C; blender, bead beater, or emulsifier), buffer constituents and concentration, pressure and membranes used during the dialysis. In order to trace which intermediates were produced by the system, the assay involved three controls and two tests with radioactive substrates. The CFE was treated with acetyl CoA, malonyl CoA and either [2-^{14}C]-malonyl CoA or [methyl-^{3}H]-*S*-adenosyl-*L*-methionine, in presence of cofactors (NADPH, FAD, and SAM). The biosynthesis of lovastatin (**1**) requires many different enzymes some of which might be removed during the filtration process. The minimum enzymatic system requires LNKS to be useful, but the complex CFE very likely includes many more enzymes, including these not involved in the biosynthesis of lovastatin (**1**). Incorporation of both [2-^{14}C]-malonyl CoA and [methyl-^{3}H]-*S*-adenosyl-*L*-methionine is a good indication that the isolated compound originates from a PKS or a FAS, although methylations can be involved in the biosynthesis of fatty acids.[2] MS or ^{1}H NMR can quickly differentiate a fatty acid from most polyketides. Unfortunately, no metabolite could be detected which showed incorporation of both ^{14}C and ^{3}H. The absence of desired activity may be the result of factors such as denaturation of LNKS, degradation by proteases or other enzymes, insolubility of the enzyme or the reactants in the buffers, and irreversible absorption on the membrane or other surfaces.

4.2 Cell free extracts of *A. nidulans*

Preparation of cell free extracts (CFEs). The method of Watanabe and Townsend for the preparation of CFEs of *Aspergillus parasiticus*[111] was initially adapted without success to *A. nidulans lovB+lovC*. Subsequently, a modification of the method of Reeves and coworkers[93] was employed to make crude extracts of active LNKS (*lovB*) and LovC protein. The method involves the use of a mortar and pestle to break the cells in presence of buffer (20 mM Tris at pH 8.0, 10% glycerol, protease inhibitors, antioxidants, quinone production inhibitor).[112] After extraction into the buffer for a few hours, the cell debris is removed by centrifugation and filtration to afford the CFE.

UV assay. The activity of the LovB protein (LNKS) is assayed by monitoring the production of pigments **41** and **42** by UV. The blank assay is prepared by adding the cofactor solution (NADPH, FAD, and SAM) to fresh CFE. The active enzyme assay solution is prepared by mixing CFE, cofactor solution, acetyl CoA and malonyl CoA. The enzyme activity is measured by following the production of the pigments **41** and **42** at 365 nm and 26 °C for 10 hours. As the only difference between the blank and the enzyme assay is the presence of substrates in the latter, and because no yellow pigments are produced by the native PKSs and FASs of the wild type *A. nidulans* (colorless cultures), any change in absorbance over time is indicative of the transformation of the acetyl CoA and malonyl CoA by LNKS into compounds **41** and **42** (λmax = 365 nm). Although *A. nidulans lovB+lovC* produces mainly 4a,5-dihydromonacolin L (**11**), the pigments **41** and **42** are also produced by LNKS alone allowing detection of the *lovB* activity. The maximum activity (maximum absorbance) of LNKS in the CFE is achieved within one hour *(A. nidulans lovB)*, after which the absorbance slowly decreases due to depletion of substrates combined with the decomposition of the products.

4.3 Cell free production of 4a,5-dihydromonacolin L (11)

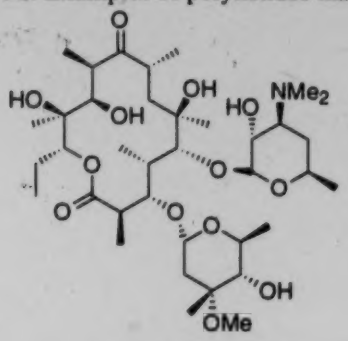
The cell free extract of the *A. nidulans lovB+lovC* strain (prepared as above) was examined to determine whether it could produce 4a,5-dihydromonacolin L (**11**). Again, no substrates were added to the blank. The enzymatic reaction was allowed to proceed overnight with acetyl CoA, malonyl CoA and the cofactors NADPH, FAD, and SAM. 4a,5-Dihydromonacolin L (**11**) was produced by the CFE with cofactors and substrates. It was purified by HPLC using the same conditions as for its original isolation, and has the same ^{1}H NMR spectra, HPLC retention time, TLC R_f, and mass spectra as the fully characterized standard. The blank was treated in a similar manner and found not to contain any detectable amount of 4a,5-dihydromonacolin L (**11**).

The production of **11** from the *A. nidulans lovB+lovC* CFE not only shows the activity of the LovC protein, but also confirms the usefulness of the UV assay for the activity of LNKS. Finally, this experiment sets the stage for additional incorporation studies with labeled NAC esters of proposed intermediates into 4a,5-dihydromonacolin L (**11**).

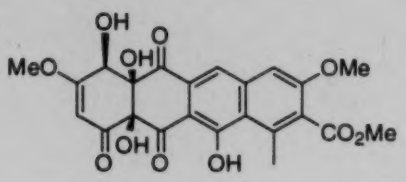
4.4 Attempted incorporation of diketide 29a into 4a,5-dihydromonacolin L (11) using CFE of *A. nidulans lovB+lovC*

In many cases where *in vivo* incorporation studies have failed, the preparation of CFEs for biosynthetic studies have shown promising results. The biosynthesis of erythromycin by *Streptomyces coelicolor*,[8] tetracenomycin C by *S. glaucescens*,[9] daunorubicin from *Streptomyces* sp. strain C5,[114] 6-methylsalicylic acid (**2**) from *Penicillium patulum*,[10] and the polyketide portion of cyclosporin A found in *Tolypocladium niveum* (Figure 32),[11] are among examples where crude bacterial or

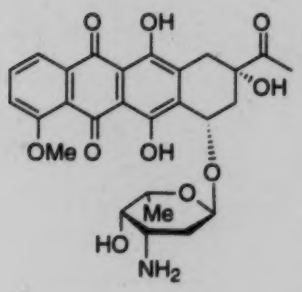
Figure 32: Examples of polyketides that have been studied using cell free extracts



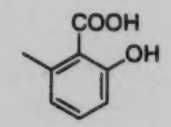
Erythromycin A



Tetracenomycin C



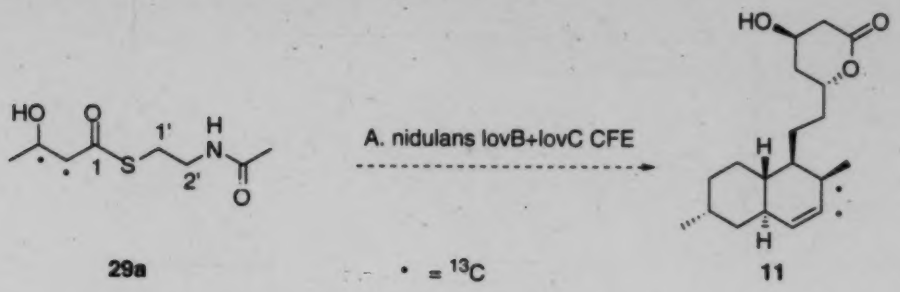
Daunorubicin



6-methylsalicylic acid

fungal PKSs have been used with success. The crude PKS enzymes present in a cell free extract may be more accessible for added precursors since the cell wall barrier is removed. Depending on the level of purification, some undesirable enzymes or metabolites may still be present in the solution, resulting in degradation of the precursors and/or of the PKSs. The diketide **29a**, as a concentrated ethanolic solution, was added to the CFE from the *A. nidulans lovB+lovC* strain in presence of malonyl CoA and cofactors (NADPH, FAD, SAM), just as was done for the *in vitro* production of 4a,5-dihydromonacolin L (**11**). In order to favor the loading of the diketide as a starter unit, no acetyl CoA was added. The ^{13}C-labeled diketide **29a** was apparently degraded by the crude enzyme preparation before intact incorporation could occur.

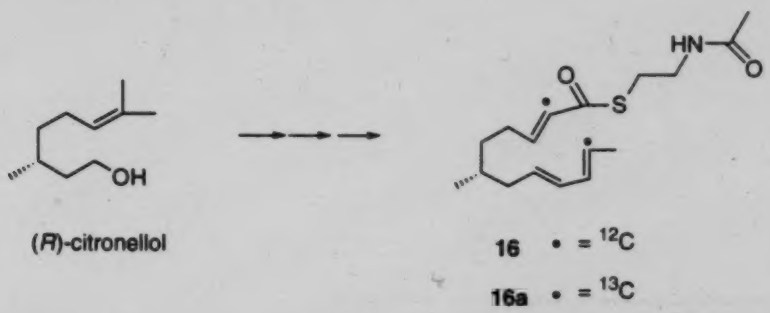
Figure 33: The diketide **29a** and 4a,5-dihydromonacolin L (**11**)



4.5 Attempted incorporation of triene 16a into 4a,5-dihydromonacolin L (11)

The ^{13}C-labeled hexaketide **16a** was synthesized as a single enantiomer from *R*-citronellol by Mr. Johan P. van den Heever, using a method previously reported (Figure 34).[46] The final triene spontaneously undergoes a Diels-Alder reaction at room temperature with a half life of 10 days in chloroform and 24 hours in water:acetonitrile:methanol at both pH 5.0 and 7.0 (see below).[46]

Figure 34: Hexaketides **16** and **16a**



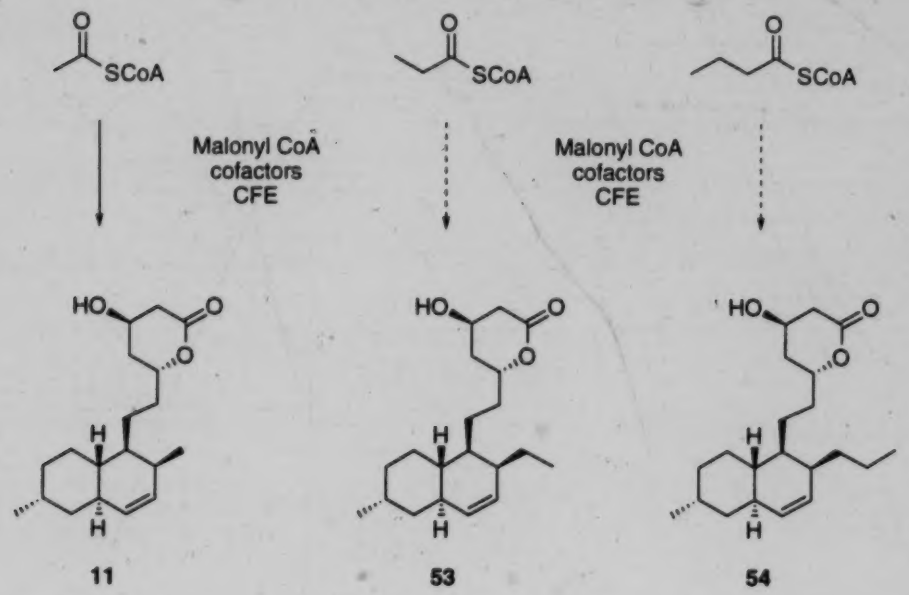
Compound **16a** was added to the *A. nidulans lovB+lovC* CFE in the presence of cofactors, malonyl CoA (no acetyl CoA), and a small portion of ethanol to help solubility. After 16 hours 4a,5-dihydromonacolin L (**11**) was isolated. MS analysis did not show

any significant ^{13}C incorporation, and the ^{13}C-labeled triene **16a** was not detected in the organic extract. This suggests that the labeled NAC ester fed may have been cyclized and/or decomposed before significant amounts could be used by LNKS and the LovC protein to make **11**.

4.6 Studies on use of propionyl CoA and butyryl CoA as starter units by the CFE of *A. nidulans lovB+lovC*

Precursor-directed biosynthesis of polyketide derivatives is a simple method to prepare novel molecules. The preparation of 12-ethyl erythromycin, which would require numerous synthetic steps, was recently achieved by the incorporation of the appropriate diketide using a mutant strain inactivated at the KS of module 1.[114] Relaxed starter unit specificity has been observed with the oxytetracycline,[115] daunorubicin,[116] and rifamycin PKSs,[117] although in some cases the flexibility of the enzymes involved at the early stage contrasts with the specifity of the later stage proteins and prevents the biosynthesis of the corresponding derivatives. In an attempt to examine the choice of starter units by LNKS and the specificity of the enzymes involved in the assembly of 4a,5-dihydromonacolin L (**11**), propionyl CoA and butyryl CoA were individually used instead of acetyl CoA as possible starter units. The addition of these two CoA thioesters in separate experiments to the CFE of the *A. nidulans lovB+lovC* strain in presence of cofactors and malonyl CoA could lead to the production of **53** and **54**, respectively (Scheme 13). However, the only additional compounds detected by HPLC were the starting material propionyl CoA or butyryl CoA. Although this suggests that LNKS selectively uses acetyl CoA as a starter unit, it could be that there is some production of **53** and **54** below the detection limit. Even the natural substrate, acetyl CoA, is used by the CFE to produce an amount of 4a,5-dihydromonacolin L (**11**) that is barely above the detection limit of our HPLC system.

Scheme 13: Precursor-directed biosynthesis of 4a,5-dihydromonacolin L derivatives



4.7 Significance

A cell free extract (CFE) prepared from the *A. nidulans lovB+lovC* strain has been shown to contain active LovB and LovC proteins. This cell free system produces 4a,5-dihydromonacolin L (**11**) from acetyl CoA and malonyl CoA in presence of cofactors. Intact incorporation of the diketide **29a** and of the hexaketide **16a** into 4a,5-dihydromonacolin L (**11**) could not be achieved apparently because of rapid degradation of the precursors. The precursor-directed preparation of 4a,5-dihydromonacolin L homologues **53** and **54** by the addition of propionyl CoA and butyryl CoA to the CFE was not possible either because of the low efficiency of this process or the specificity of

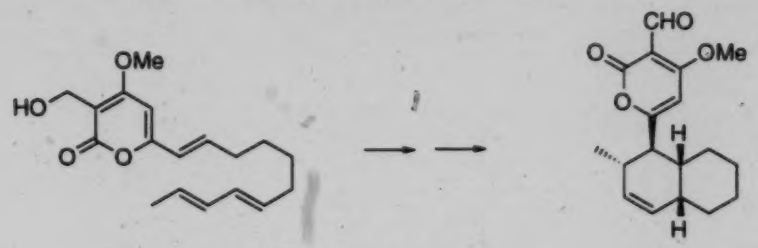
the LovB+LovC system for its natural starter unit. The failure of these experiments suggest that undesirable enzymes and/or metabolites present in the CFE still interfere with the biosynthetic studies. In CFEs, enzymes or metabolites that do not come into contact with each other in a normally functioning cell, may do so with undesired results when the membranes and the other structural units are removed. The unsuccessful *in vivo* and *in vitro* experiments for the elucidation of the PKS transformations suggests that further purification of LNKS is necessary for incorporation studies. Purification of both LNKS and LovC proteins followed by recombination should lead to a clean and efficient production of 4a,5-dihydromonacolin L (**11**).

5. Enzyme-Catalyzed Diels-Alder Reactions

The first enzyme was discovered in 1933 by Payen and Perzon.[118] Three years later Schwann extracted the active component of gastric juice and called it pepsin.[118] Today, an enzyme-catalyzed equivalent is known for many types of chemical reaction.[113] These include a large variety of oxidations, reductions, hydrolyses, condensations, and isomerizations.[120] In addition to lowering the activation energy for many processes such that they proceed readily at physiological conditions, enzymes are usually highly stereospecific and produce single enantiomers.[113] In spite of their excellent recognition of natural substrates, certain enzymes can accept alternative "unnatural" substrates, and this makes them extremely useful reagents in organic synthesis.[113]

Despite many proposals of involvement of "Diels-Alderases" in biosynthesis,[83] prior to our work only one such enzyme has been detected[84] and partially purified.[89] Oikawa, Ichihara and coworkers have characterized an oxygenase that oxidizes the primary alcohol of prosolanapyrone II (**23**) thereby activating an exo-selective Diels-Alder reaction on the enzyme surface to produce chiral solanapyrone A (**17**).

Figure 35: Prosolanapyrone II (**23**) and solanapyrone A (**17**)



23 **17**

It would be very interesting to combine the importance of Diels-Alder reactions in asymmetric synthesis with the enormous power of enzymes into a Diels-Alderase. In order for this new type of enzyme to prove useful in synthesis, a wide range of substrates

should be tolerated by the enzyme. The LovB protein appears a possible candidate for a Diels-Alderase based on the proposed biosynthetic pathway.

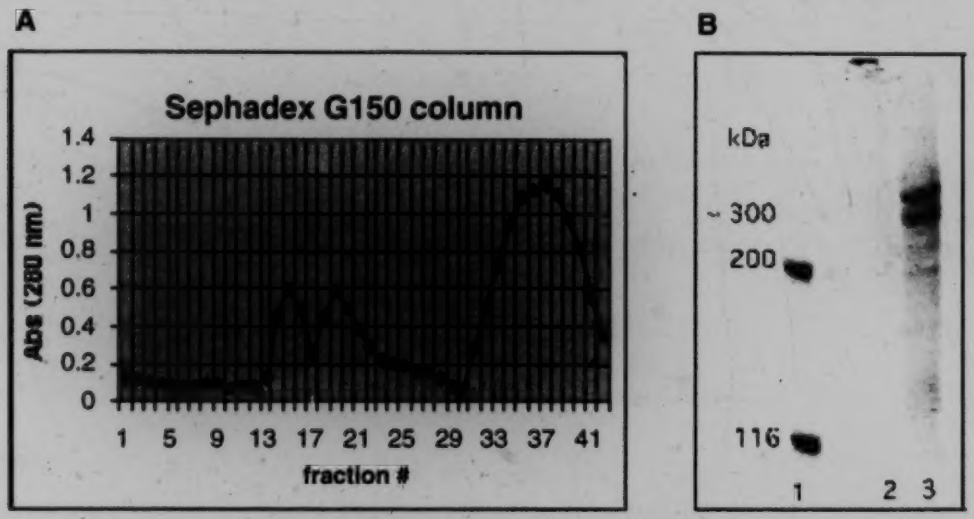
5.1 Lovastatin nonaketide synthase (LNKS or LovB protein)

Since the biosynthesis of 4a,5-dihydromonacolin L (**11**) requires both LovB and Lov*C* proteins, either enzyme, or even their combined activity, may catalyze the proposed Diels-Alder reaction. The *lovB* gene encodes for a protein of approximately 335 kDa (LNKS or its precursor), whereas *lovC* encodes an enzyme of 39 kDa molecular weight (see Table 1). Although the latter protein seems to contain only an ER domain, the LNKS is predicted to contain the six common PKS active sites, KS, AT, DH, ER, KR and ACP. In addition, it has a MeT region (not common amongst PKSs) and a rare replacement of the *C*-terminal TE domain by an approximately 500 amino acid-long segment, possibly involved in product transfer or cyclization. The extensive functionality of the LovB compared to the LovC protein and its unusual *C*-terminus, make LNKS (*lovB*) more likely to catalyze a Diels-Alder reaction. For these reasons and because of the availability of the *A. nidulans lovB* strain containing the overexpressed gene, LNKS was purified.

5.2 LNKS enzyme purification

The LovB protein was purified using a modification of the method of Reeves and coworkers.[93] The *A. nidulans lovB* CFE prepared as described above, was concentrated and purified by size exclusion column chromatography (SEC). As shown by SDS-PAGE (Figure 36), the first fraction of the SEC (lane 2) contained a protein of about 600

Figure 36: Elution curve and SDS-PAGE of the pooled protein samples collected during size exclusion chromatography in the purification of LNKS



A: UV (280 nm) elution profile of the fractions collected during the purification of LNKS by size exclusion chromatography on Sephadex G-150. The first pooled protein sample contained fractions 13-16 and the second contained fractions 18-22.
B: SDS-PAGE was performed on 5% Bio-Rad Ready Gels at 150V for 80 min and stained with Coomassie Blue. Lane 1 is a high range molecular weight protein standard. Lane 2 is the first pooled protein sample containing a protein of about 600 kDa. Lane 3 is the second pooled protein sample which is believed to consist of mainly the desired protein of predicted molecular weight of 335 kDa.

kDa. The second protein fraction consisted of mainly the desired protein based on its predicted molecular weight of 335 kDa (lane 3). The minor protein observed in the latter is believed to arise from decomposition of the target protein during precipitation since it is not observed in the crude extract. Finally, lane 1 consists of high molecular weight protein standards.

The pooled protein samples were precipitated with ammonium sulfate and collected by centrifugation. The LNKS enzyme activity could be monitored as described for the CFE, and is located in the second poooled protein sample (i.e. lane 3), in agreement with SDS-PAGE.

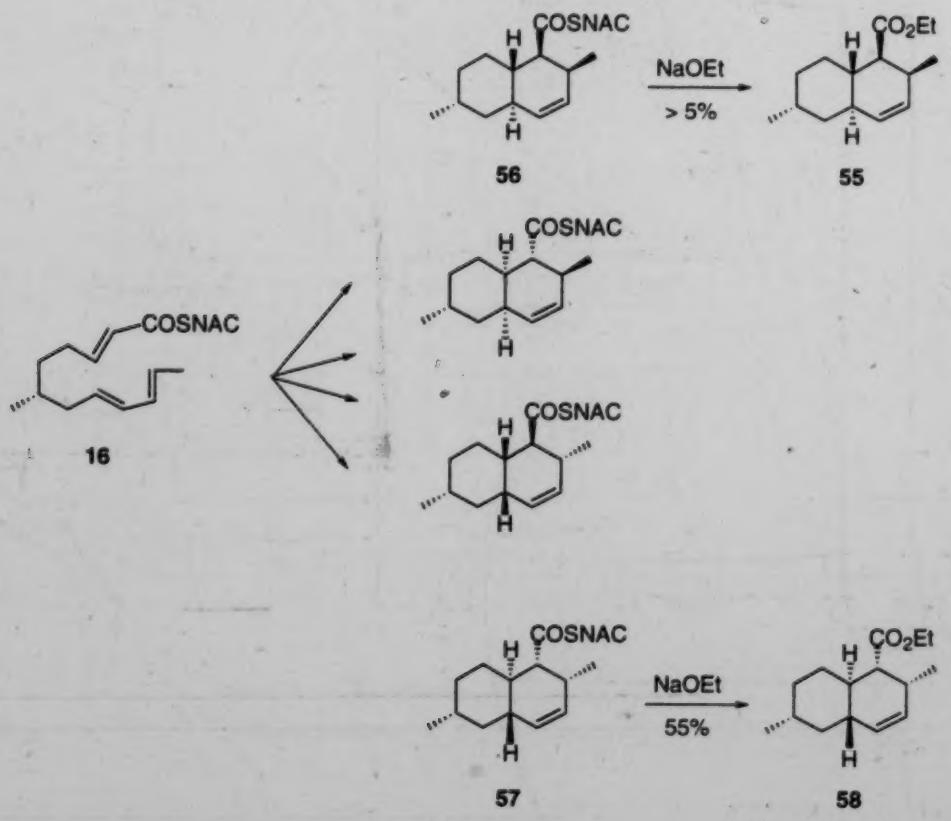
5.3 LNKS-catalyzed Diels-Alder reaction of 16

The degradation of the ^{13}C-labeled NAC esters during both *in vivo* and CFE assisted incorporation experiments suggests that the elucidation of 4a,5-dihydromonacolin L (**11**) biosynthesis may be facilitated if the enzymes involved are purified. With the purified LNKS in hand, it seemed easier to verify whether or not the protein could catalyze the Diels-Alder reaction proposed during the biosynthesis of lovastatin (**1**). The Diels-Alder reaction is believed to occur at the hexaketide stage, when the dienophile is activated by the adjacent thioester formed by loading on the enzyme. Compound **16** (Figure 34 and Scheme 14) is designed to mimic the enzyme-linked hexaketide, and therefore seemed an ideal candidate to explore the possibility of a LNKS-catalyzed Diels-Alder reaction. A solution of unlabeled triene **16** in ethanol was added to a buffer solution of the enzyme prepared as above, and was allowed to react on an orbital shaker at 25°C for 16 h. The mixture was then either purified by HPLC or used directly in the transesterification described below, to confirm the structure of the product(s). There are four possible diastereoisomeric products of the Diels-Alder reaction of **16** (Scheme 14, see also Scheme 3). It was shown by our group that the Diels-Alder adducts of **16**, or of the corresponding acid or ester, produced under thermal or Lewis acid ($EtAlCl_2$) conditions do not have the stereochemistry required to lead to lovastatin (**1**). This was proven by the independent stereoselective synthesis of the ethyl ester derivative **55** (Scheme 14) by Dr. David J. Witter.[46] The crude LNKS-catalyzed Diels-Alder reaction mixture of **16** was extracted with chloroform and evaporated. This crude organic extract was analyzed by HPLC and purification of the sample showed three compounds with the molecular weight expected for the Diels-Alder adducts of **16**. Two of these had retention times that matched the Diels-Alder adducts resulting from spontaneous cyclization as prepared Dr. David Witter.[46] In a separate experiment aimed at identifying the third

diastereoisomer, the organic extract was treated with sodium ethoxide in dichloromethane and ethanol. The reaction produced again three compounds with the expected molecular weight. The two products of spontaneous cyclization (*endo* and *exo*) of the ester analogue of **16** were observed as well as a compound with the same properties as **55** (IR, MS, TLC and HPLC). Since **56** is the only diastereoisomer of the 4 possible that could produce **55** by reaction with sodium ethoxide, this confirms our hypothesis that a LNKS-catalyzed Diels-Alder reaction is involved during the biosynthesis of lovastatin (**1**) by fungi. Epimerization is not expected to have occurred based on two facts. The starting material required to give **55** by epimerization at C-1 is not a possible Diels-Alder adduct. Also, the sodium ethoxide reaction was tested with the model compound **57** and shown to produce only **58** and recovered starting material (Scheme 14). An alternative synthesis and the full characterization of **58**, used here as a standard, has been reported by our group.[46] Based on the difference in diastereoselectivity between the chemical and the enzymatic reaction, LNKS must catalyze the Diels-Alder reaction of **16**. Since LNKS catalyzes cyclization of **16** to give the NAC ester product **56**, covalent attachment to the enzyme is probably not necessary.

This experiment proves that the major purified PKS involved in the biosynthesis of lovastatin (**1**), LNKS or the LovB protein, does catalyze a Diels-Alder reaction in the absence of other substrates and cofactors. Although, Diels-Alderases have been suggested for the assembly of many polyketides,[83e] LNKS is the first experimentally proven case of such an enzyme activity. This is also the first example of a Diels-Alderase which has been purified.[84e] LNKS and partially purified solanapyrone synthase[84e] are

Scheme 14: Demonstration of LNKS-catalyzed Diels-Alder reaction



two very different enzymes. The former is a multifunctional enzyme of especially large molecular weight (335 kDa), whereas the latter is an oxidase of molecular weight approximately 55 kDa.[84e]

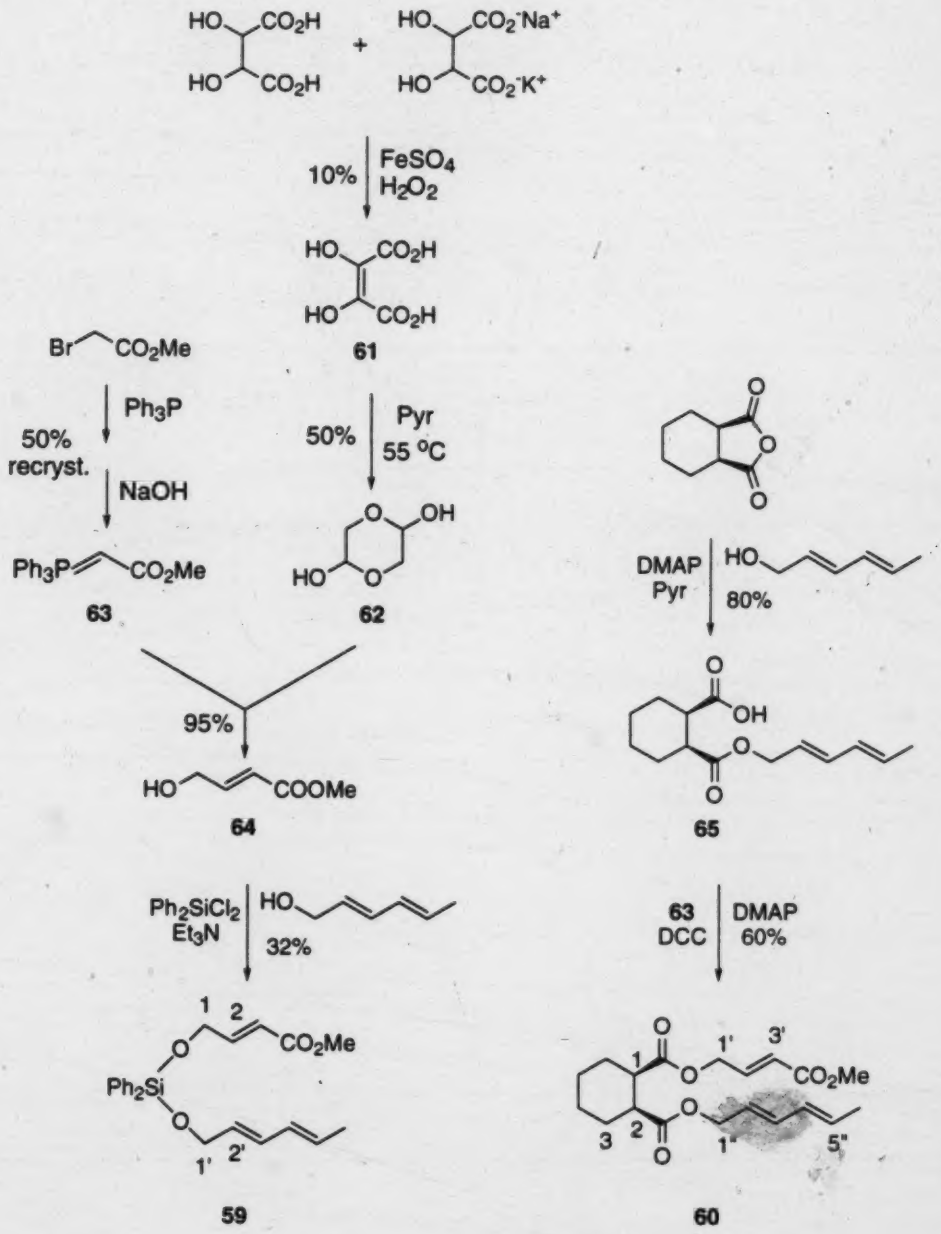
Purification of the LovB protein will allow detailed characterization of properties and substrate specificity. Once the LovC protein is also purified, it should allow conversion of advanced intermediates to 4a,5-dihydromonacolin L (**11**).

5.4 Synthesis of alternative trienes for exploration of substrate specificity of LNKS

A series of tethered diene-dienophile systems were synthesized to explore whether LNKS and several other enzymes can catalyze their intramolecular Diels-Alder cyclization. Compounds **59** and **60** represents readily accessible and versatile candidates for such a study. Both the diene and the dienophile sections can easily be varied and connected via the silyl or ester homologues. Silyl derivatives **59** could be prepared by a modification of the method of Ainsworth *et al.*[52a] (Scheme 15). Oxidation of tartaric acid to dihydroxymaleic acid (**61**),[174] followed by treatment with pyridine gives glycolaldehyde (**62**).[175] Wittig reaction between **62** and the triphenylphosphorane **63** (prepared from methyl 2-bromoacetate) affords dienophile **64**. Dichlorodiphenylsilane readily tethers the commercially available diene (*E,E*)-2,4-hexadien-1-ol with the dienophile **64** to afford compound **59**. An alternative tether that can be used is *cis*-hexahydrophthalic anhydride.[52d] The preparation of intermediate **65** proceeds in 80% yield by ring opening of the anhydride with (*E,E*)-2,4-hexadien-1-ol in presence of 4-dimethylaminopyridine (DMAP) and pyridine. The target triene **60** is produced by the reaction of **65** with the dienophile **64** in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and DMAP.

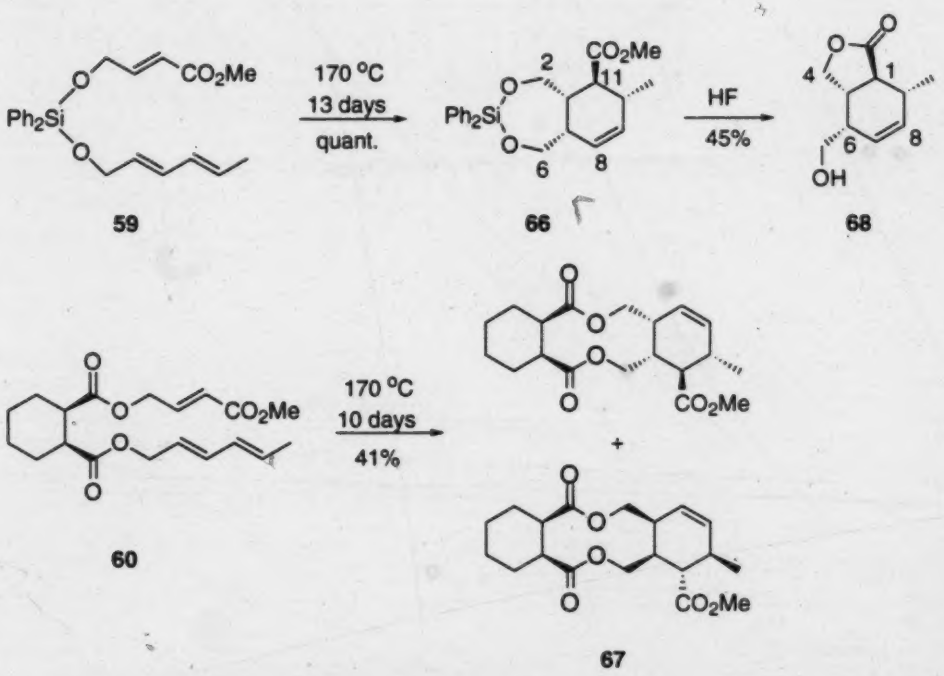
Compounds **59** and **60** were tested for chemical Diels-Alder reactivity in the same manner as described by Ainsworth *et al.*[52a,b,c] and Craig *et al.*[52d] (Scheme 16). The reactions proceed very slowly in toluene. The transformation of compounds **59** and **60** to **66** and **67**, respectively, require more than a week in toluene at 170 °C and in a sealed tube. The structure of the Diels-Alder adduct **66** can be confirmed by cleavage of the

Scheme 15: Synthesis of trienes **59** and **60**



silyl tether and spontaneous lactonization to compound **68**. Finally, the stability of both trienes in aqueous conditions was followed by ^{1}H NMR spectroscopy in D_2O:DMSO-d_6. The very low solubility of compounds **59** and **60** in water requires about 30% DMSO to allow detection by a 360 MHz instrument. The ^{1}H NMR spectra were unchanged after one week indicating that no detected cyclization occurs in this solvent system at room temperature.

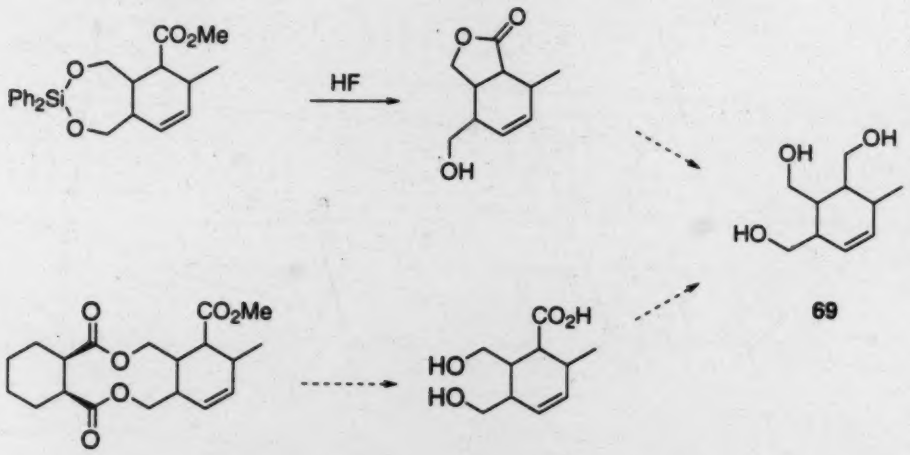
Scheme 16: Thermal Diels-Alder reaction of compounds **59** and **60**



Since the Diels-Alder adduct of **59** is unstable, a possible alternative assay leading to the same probe compound **69** for both the Diels-Alder adducts of **59** and **60** was investigated (Scheme 17). After both Diels-Alder reactions are completed as before, the tethers may be removed either with HF (compound **59**) or with lithium hydroxide (compound **60**). Subsequent reduction, with sodium borohydride, lithium aluminum

hydride, or borane, should lead to the triol **69**. Quantification of the different isomers of **69** would reveal the stereoselectivity of the reaction. Unfortunately, this assay did not prove useful because of the high aqueous solubility of the triol **69** in water that complicated its purification.

Scheme 17: Suggested alternative assay for Diels-Alder adducts of compounds **59** and **60**



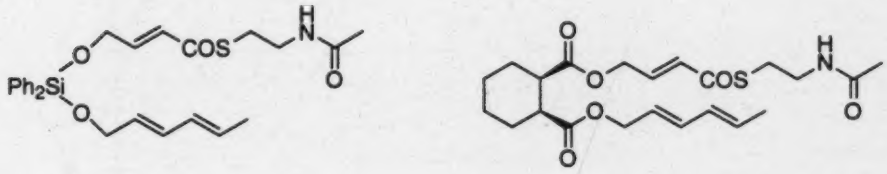
Attempted Diels-Alder reaction assay of 59 and 60 in presence of LNKS. The possible reactions of **59** and **60** catalyzed by LNKS were done separately as follows. The protein pellet obtained from the purification of LNKS was redissolved in buffer, and this solution was diluted with water, and ethanol to help dissolve the hydrophobic trienes. Compounds **59** and **60** were added, and the mixtures were left to react for three days. Work up only recovered **59** and **60** and no cyclization products could be detected.

Several factors could potentially explain why LNKS does not catalyze the Diels-Alder reaction of **59** and **60**. The two trienes have structures and properties that differ greatly from the natural substrate of LNKS. They are insoluble in water and also much larger than the lovastatin derivatives. The enzyme may be quite substrate specific, and

recognition of the hydrophilic thioester linkage (i.e. NAC) may be required. Since LNKS catalyzes cyclization of **16** to give the NAC ester product **56**, covalent attachment to the enzyme is probably not necessary.

To enhance their resemblance to the acceptable substrate of LNKS, the syntheses of the NAC thioesters **70** and **71** were attempted (Figure 37).

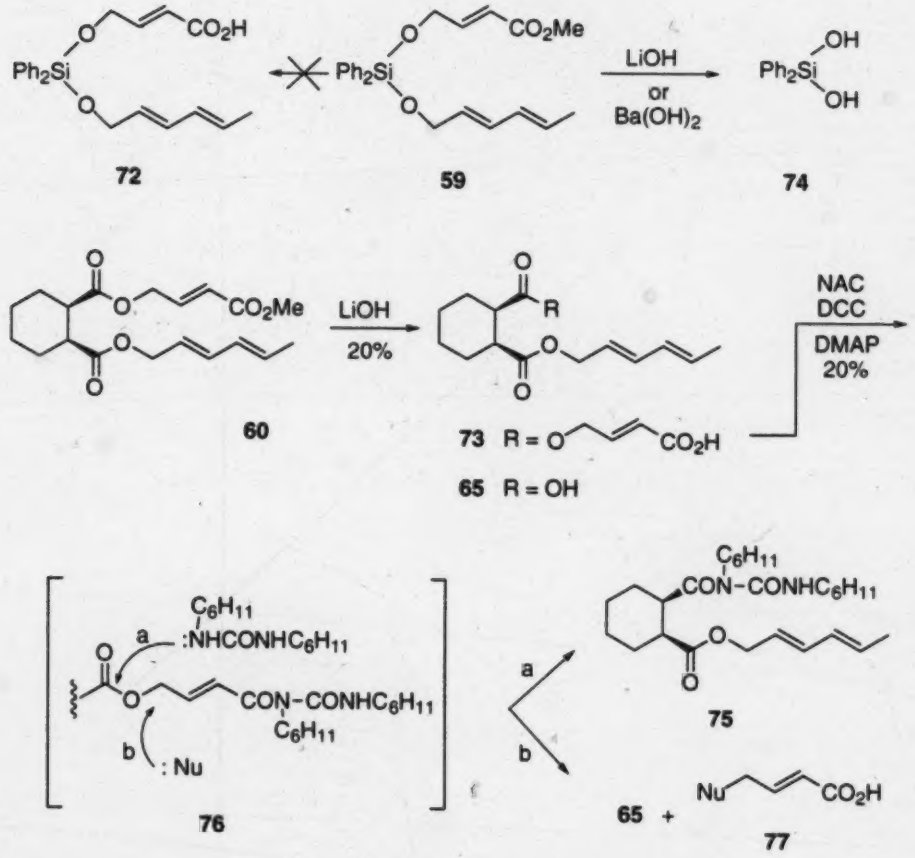
Figure 37: The NAC thioesters **70** and **71**



70 **71**

Coupling to NAC requires the preparation of the two corresponding acids **72** and **73** (Scheme 18). Hydrolysis of the ester **59** using either lithium hydroxide or barium hydroxide leads to quantitative removal of the tether to afford dihydroxydiphenylsilane (**74**), (*E*,*E*)-2,4-hexadien-1-ol and methyl 4-hydroxy-2-butenoate (**64**). Alternatively, the selective hydrolysis of the methyl ester of compound **60** by lithium hydroxide gives 20% of the desired acid **73** as well as the shorter acid **65** as the major product. Treatment of NAC with the acid **73** in presence of DCC and DMAP generates the acid **65** and compound **75**, which may result from the rearrangement of intermediate **76**. Although the exact reason for this remains unidentified, it may be that activation of the acid results in a highly activated allylic system that is prone to nucleophilic attack at the ester carbon to give **65** and a species like **77**.

Scheme 18: Attempted preparation of compounds **70** and **71**



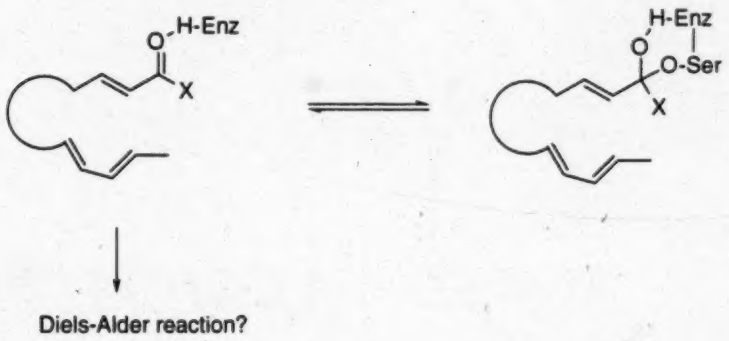
5.5 Investigation of subtilisin Carlsberg as a possible Diels-Alderase

Compounds **59** and **60** were tested for Diels-Alder cyclization with other enzymes, including the serine protease subtilisin Carlsberg (EC 3.4.21.14). The physiological role of this enzyme is to hydrolyse water soluble proteins.[121] The catalytic reaction takes place through the formation of an intermediate acyl-enzyme resulting from

nucleophilic attack of the serine hydroxyl group to the carbonyl of the substrate and expulsion of the leaving group. The acylated enzyme is subsequently deacylated by attack of a nucleophile from the reaction medium.

As a result of its relaxed substrate specificity, its enantioselectivity, and its commercial availability, subtilisin Carlsberg has been used extensively for hydrolysis, esterifications and transesterifications in either aqueous or organic media.[113,122] This enzyme has also been used in molecular imprinting studies,[123] and cross-linked enzyme crystal preparation.[122,124] The poor water solubility of compounds **59** and **60** indicated the use of organic media. The properties of subtilisin Carlsberg in organic solvents have been studied by many groups.[125,126] Low water systems have been showed to increase the activity and selectivity of this protease. The method reported by Zaks and Klibanov[125] was adopted. Thus, the pH adjustment of subtilisin Carlsberg was effected by lyophilization of a solution of the enzyme in phosphate buffer at pH 7.8. The white powder obtained was used in *n*-octane to transesterify the methyl esters **59** and **60** separately with methanol. Since proteases are believed to catalyze reactions by activation of the carbonyl functionality[121] and Diels-Alder reactions are facilitated by this process,[47] subtilisin Carlsberg could potentially accelerate the cyclization of **59** and **60** as the methoxy group is exchanged (Scheme 19). Unfortunately, the enzymatic reaction does not facilitate the cycloaddition as expected and the starting material was recovered in both cases. Many factors can account for this, including unfavorable conformation of substrate at the active site and insufficient and interaction to overcome the high activation energy barrier to cyclization.

Scheme 19: Activation of carbonyl funtionalities by serine proteases



5.6 Investigation of carbonic anhydrase as a possible Diels-Alderase

When carbon dioxide is dissolved in water it is slowly hydrated to carbonic acid which spontaneously ionizes according to the equation given in Scheme 20. Hydration occurs in the pH range 6.5 to 10.0, whereas the reverse dehydration takes place in the pH range 5.5 to 7.5. This reversible reaction is catalyzed by many buffers including phosphate, veronal, chromate, cacodylate, borate, selenite, and also by the enzyme carbonic anhydrase (EC 4.2.1.1) which catalyzes both directions of the reaction equally.[127]

Scheme 20: Reaction catalyzed by carbonic anhydrase

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The solvation of carbon dioxide in water is critical to mammalian respiration. Its transformation to carbonic acid lowers the pH of internal fluids, directly affecting the central nervous system which controls breathing. Without sufficient CO_2, respiration ceases and death rapidly follows, yet too much causes hyperventilation which can result

in dizziness or a comatose state. Carbonic anhydrase is found in most cells, especially in the blood, in the lungs, and in the central nervous system. Mammalian red blood cells are the richest source of this enzyme.

Carbonic anhydrase is very specific for the reaction of CO_2 with H_2O. It is a zinc dependant enzyme[128] of ~ 30 kDa molecular weight, with one atom of Zn per molecule. The Zn (II) is believed to activate the carbonyl of the substrate. The mammalian enzyme is inhibited by cyanide, sulfide, and azide, whereas the plant enzyme is insensitive to cyanide. Some anions present in the blood have inhibitory effects on carbonic anhydrase (e.g. I^-, NO_3^-, and Br^-). Although this enzyme is very specific for carbon dioxide and carbonic acid, certain larger molecules such as aryl sulfonamides bind to Zn and inhibit at nanomolar concentrations. If a triene can enter the active site, the zinc atom may coordinate with the dienophile carbonyl and allow activation in the chiral environment of the enzyme, thereby leading to an enantioselective Diels-Alder reaction. A possible problem could be the long (15 Å)[128] narrow active site of carbonic anhydrase which may favor an extended conformation of such substrates or block entry of bulky compounds.

Once again, the low water solubility of substrates **59** and **60** suggested the use of organic media. Since only aqueous enzymatic reactions have been reported for carbonic anhydrase, the method of Klibanov and coworkers[129] for alcohol dehydrogenase was adapted for both carbonic anhydrase and alcohol dehydrogenase (see below). Carbonic anhydrase from bovine erythrocytes was immobilized on glass beads and suspended in isopropyl ether containing 0.02% buffer. The enzymatic reaction was initiated by the addition of either **59** or **60** under argon, and followed by UV at 240 nm. After gentle shaking for two days, the starting materials **59** and **60** were each recovered unchanged. Since the enzyme was shown to be active under these conditions, the lack of reactivity is probably due to compounds **59** and **60** not being able to enter the narrow active site.

5.7 Investigation of alcohol dehydrogenases from horse liver or from Baker's yeast (HLADH and BYADH) as possible Diels-Alderases

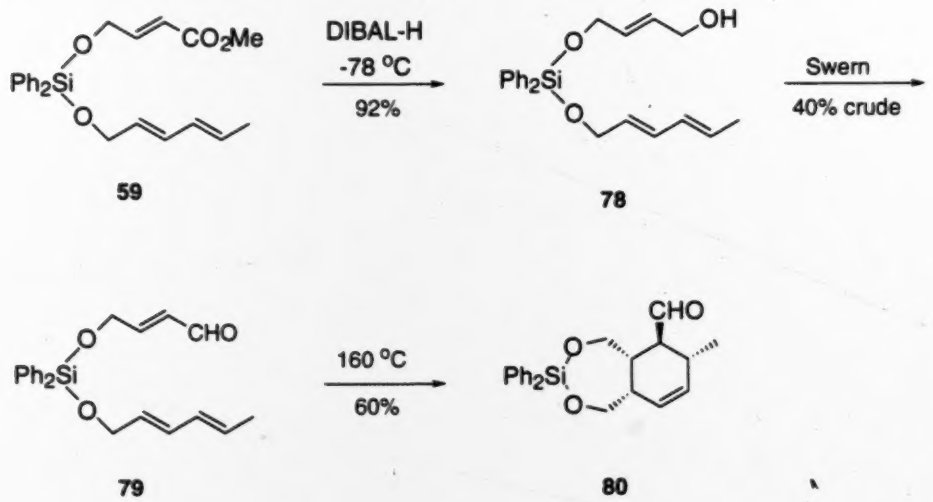
Alcohol dehydrogenases (ADH, EC 1.1.1.1) are the enzymes that catalyse the reversible oxidation of alcohols to aldehydes or ketones using the cofactor NADP/NADPH.[130] Generally, ADH is active as a homodimer in which each monomer contains two zinc ions, one of which is essential in catalysis, and one that has been shown to play a structural role.[131] Each monomer is made of two subunits of different size separated by a cleft. The smaller unit has a NADP/NADPH binding site, whereas the bigger unit comprises the two zinc ions and the active site pocket. The enzyme has been extensively studied and the crystal structure is known for several complexes.[131,132] Horse liver and Baker's yeast are the most common source of ADH. Commercially available HLADH usually contains three dimeric isozymes: EE, ES, and SS (E for ethanol and S for steroids).[133] BYADH is commonly used as a "cocktail" of dehydrogenases and its application is fairly simple, lacking all the technical difficulties that are usually associated with handling oxidoreductases in pure form.[133] Molecular weights of 73 kDa for HLADH and of 150 kDa for BYADH have been measured.[127] These zinc-dependent enzymes rely on free SH groups for activity, and therefore are inactivated by SH-inhibiting agents, and by salts such as $AgNO_3$, $HgCl_2$ or $CaCl_2$.[127]

There have been numerous reports on the use of alcohol dehydrogenase to transform a large variety of substrates, both in aqueous and organic media. In addition to ethanol, the enzyme reacts with higher aliphatic alcohols like amyl alcohol, ethylene glycol, or vitamin A.[130] A model of the steric requirements and topography of the substrate binding region has been proposed by Jones and coworkers,[133] which allows prediction of whether a given alcohol will be a substrate. The growing use of ADH has triggered the development of cofactor regeneration methods.[134,135]

As mentioned above, Diels-Alder reactions are facilitated by electron-poor dienophiles and electron-rich dienes. ADH can transform an allylic alcohol (poor dienophile) into an unsaturated aldehyde (good dienophile) which may potentially cyclize rapidly on the enzyme surface. Like carbonic anhydrase, ADH also contains a zinc atom that could in principle accelerate the Diels-Alder reactions of esters **59** and **60** by Lewis acid catalysis.

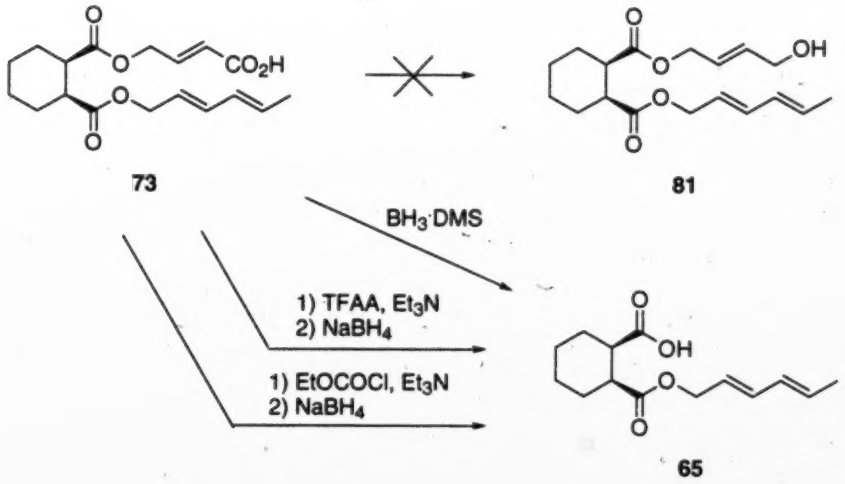
Synthesis of triene alcohols. Reduction of the methyl ester **59** with DIBAL-H proceeds in 92% yield to the allylic alcohol **78** (Scheme 21). Chemical oxidation of the alcohol **78** was performed using Swern oxidation[136] in order to investigate the stability of the corresponding aldehyde **79**. The triene **79** undergoes Diels-Alder cyclization to give 60% of the *exo* product **80**.

Scheme 21: Synthesis of alcohol **78** and reactivity of the aldehyde **79**



Numerous attempts to reduce the acid **73** to the allylic alcohol **81** were unsuccessful (Scheme 22). Treatment with borane-dimethyl sulfide[137] in THF at -50 °C and subsequent warming to 20°C yields the acid **65** after work up by hydrolysis of the ester at C-1. Sodium borohydride reduction of the mixed anhydrides prepared from either trifluoroacetic anhydride (TFAA) or ethylchloroformate in presence of triethylamine, also afford the same acid **65**.

Scheme 22: Attempted synthesis of alcohol **81**



Enzyme tests. The alcohol **78** and the two esters **59** and **60** were each independently tested with both HLADH and BYADH using two different methods. Thus each ADH was immobilized on glass beads and exposed in isopropyl ether and 1% buffer to the individual substrates **59**, **60**, and **78**.[129] In a second method, substrates **59**, **60**, or **78** were independently added to solutions containing: ADH, NAD, buffer, and DMF. In all cases, unchanged starting material was recovered after more than a week, and no product could be detected.

5.8 Investigation of tyrosinase as a possible Diels-Alderase

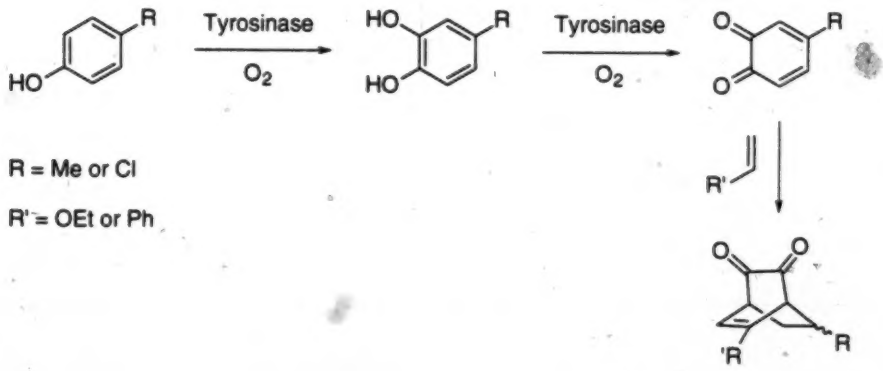
Tyrosinase (EC 1.14.18.1) catalyzes the two step oxidation of tyrosine to *ortho*-DOPAquinone in the presence of oxygen (see Scheme 5).[91] The activities of this copper-dependant enzyme at each step can be determined independently. The monophenolase or cresolase activity refers to the transformation of tyrosine to DOPA, whereas the diphenolase or catecholase activity characterizes the conversion of the catechol to *ortho*-DOPAquinone. The product of the monophenolase activity, DOPA, acts as a cofactor for tyrosinase.[138] Isolation of this enzyme is routinely done from melanocytic tumor cells or from mushrooms. Fungal and mammalian tyrosinases have molecular weights of 46 and 67 kDa, respectively. These enzymes are inhibited by a large number of copper chelating agents such as azide, cyanide and β-mercaptoethanol, as well as by carbon monoxide.[138] The quinone product and many substrate analogues are also inhibitors of this oxidase.

In the cell, molecules of *ortho*-DOPAquinone are quickly assembled into polyphenol biopolymers, like melanins, with a complex structure and color varying from yellow to black. Their biosynthesis can be observed on the surface of a cut apple, potato, or banana exposed to air. Tyrosinase is concentrated in specialized pigment-producing cells called melanocytes. The color of mammalian skin, hair and eyes is determined by a number of factors, the most important of which is the degree of melanin pigmentation or the distribution of melanocytes. As a result, tyrosinase is an important enzyme in the food industry and in melanoma studies. In nerve cells, the anti-Parkinson drug DOPA is prepared by a different enzyme termed tyrosine hydroxylase (EC 1.14.16.2).

The interesting domino reaction sequence triggered by tyrosinase reported by Müller and Waldmann[92] involves intermolecular Diels-Alder reactions of *in situ* produced *ortho*-quinones (electron-poor) with two electron rich dienophiles (Scheme 23). Their products were all racemic, indicating that although tyrosinase does catalyze the oxidation, it does not influence the Diels-Alder step. It seemed that intramolecular cyclizations could

occur on the enzyme surface because of their faster rate compared to intermolecular processes. The activity of tyrosinase has been studied both in aqueous[139] and in organic

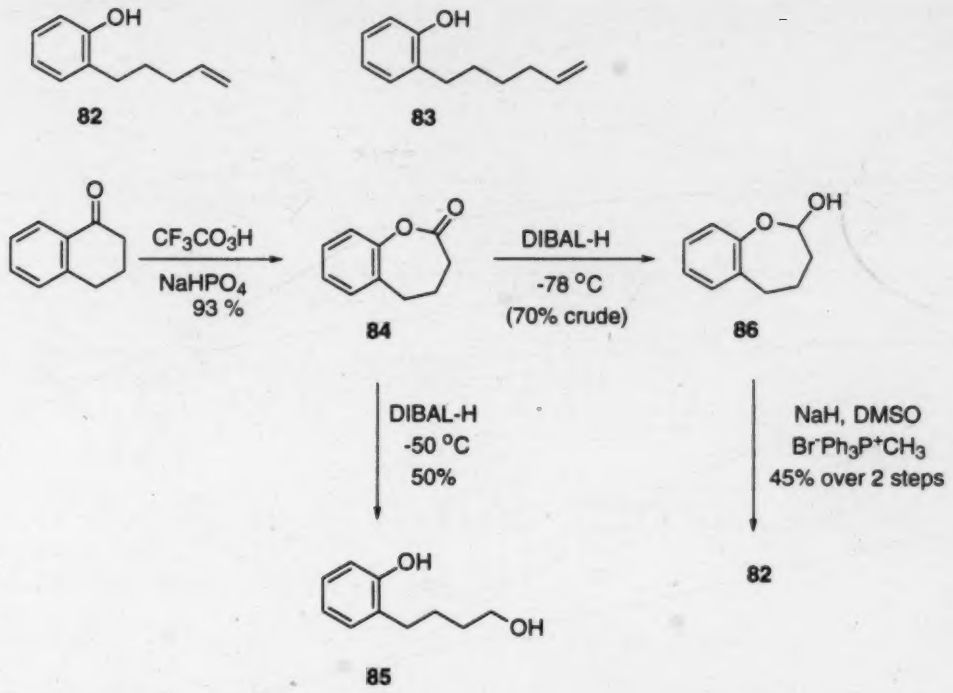
Scheme 23: Enzyme-initiated hydroxylation-oxidation-Diels-Alder reaction cascade



media.[140,141] Since the position of the side chain may be critical to the enzyme activity, both *ortho*- and *para*-substituted phenols were examined as possible substrates for tyrosinase.

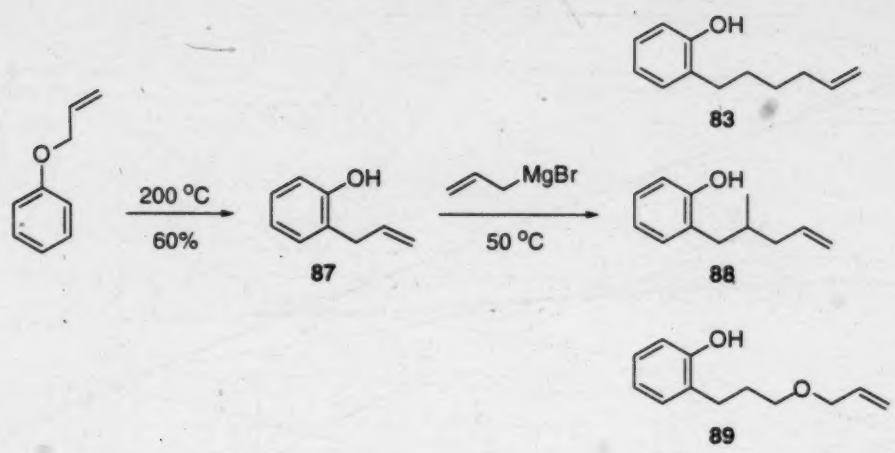
***ortho*-Phenols.** The first two targets were the *ortho*-phenols **82** and **83**. The synthetic scheme used by Yates and Macas[142] could be modified for the preparation of compound **82** (Scheme 24). A Baeyer-Villiger reaction on α-tetralone affords the ε-lactone **84** in 93% yield. DIBAL-H reduction of the lactone **84** at -50 °C yields diol **85**. When the same reaction is done at -78 °C, compound **86** is obtained as an unstable mixture of aldehyde and hemi-acetal. This crude mixture can be treated directly with the Wittig reagent derived from methyltriphenylphosphonium bromide to afford the target compound **82**.

Scheme 24: Synthesis of *ortho*-phenol **82**



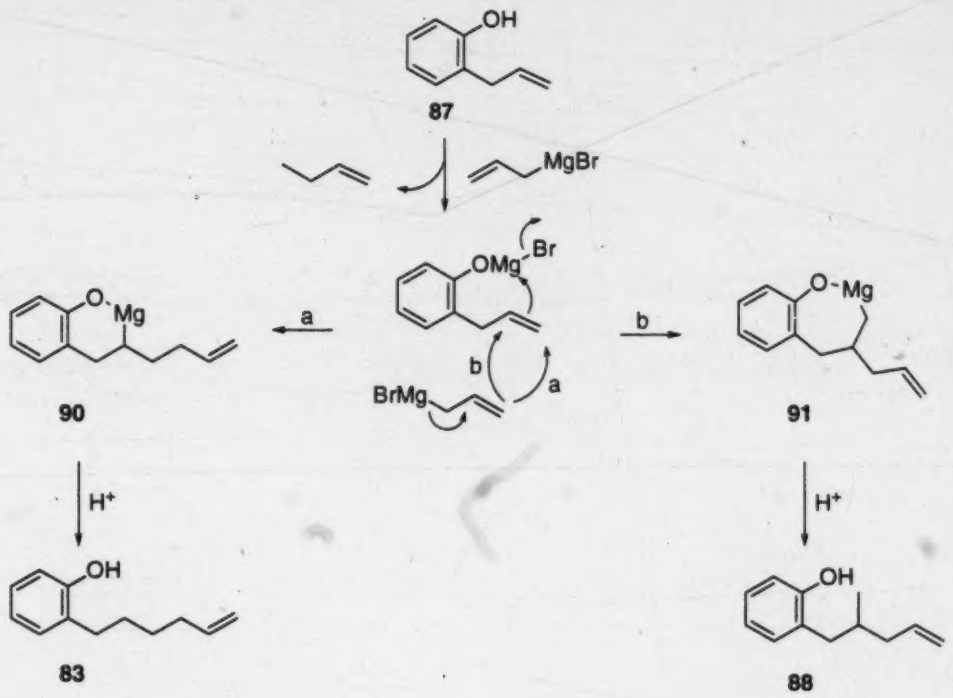
Richey and Domalski[143] reported a short synthesis of the target compound **83**, using an interesting Grignard-like reaction. In the first step, a Claisen rearrangement at 200 °C converts allyl phenyl ether to 2-allylphenol (**87**) (Scheme 25). In the critical step, the reaction of compound **87** with allylmagnesium bromide at 50 °C gives three compounds **83**, **88**, and **89** in a 7:2.4:1 ratio, respectively. These compounds were proposed to be formed by the mechanisms shown on Scheme 26. The Grignard reagent is believed to first deprotonate the phenol and then to attack the double bond at either position to afford compounds **83** and **88** after hydrolysis of the cyclic intermediates **90** and **91**. Unfortunately, compounds **83** and **88** could not be easily separated using a variety of processes. The minor component, allyl ether **89**, may arise by attack of allylic alkoxide formed from allyl alcohol contamination of the allyl bromide (Scheme 26).

Scheme 25: Synthesis of the *ortho*-phenol **83**



The reactivity of the *ortho*-phenol **82** in the presence of tyrosinase was tested as follows. Non-aqueous conditions are often preferable in syntheses using tyrosinase because water is known to accelerate the polymerization of the *ortho*-quinone products. The methods described by Müller and Waldmann,[92] and by Burton and coworkers[140] for reactions of tyrosine in organic medium were adapted. The enzyme was immobilized on glass beads at pH 7.0. The enzymatic reaction was started by the addition of immobilized tyrosine to a solution of substrate **82** in dry chloroform and buffer. The reaction was followed by TLC for up to two weeks. The enzyme was found to be active under these conditions. However, compound **82** was not transformed by the enzyme, suggesting that the catalyst may not tolerate *ortho*-substituents. This hypothesis was subsequently confirmed by substrate specificity studies by Krol and Bolton.[144]

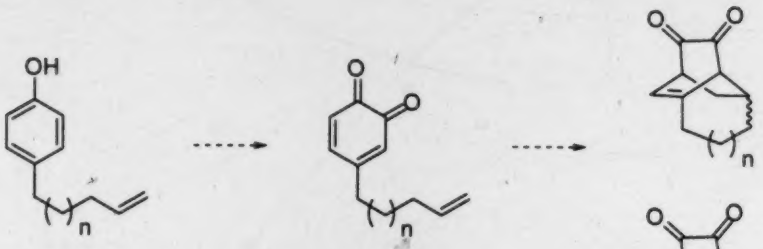
Scheme 26: Proposed mechanism[143] for the formation of compounds **83** and **88**



***para*-Phenols.** The next targets were *para*-phenolic compounds **92**, **93**, and **94**. Upon oxidation by tyrosinase, the corresponding expected *ortho*-quinones **95**, **96**, and **97** may undergo intramolecular Diels-Alder reactions to give a maximum of four possible products each (Scheme 27).

In 1973, Moreau and Rouessac[145] reported a synthesis of compound **92** from 4-methoxyphenylacetic acid. Their method was modified as in Scheme 28. Reduction of 4-methoxyphenylacetic acid with lithium aluminum hydride gives the alcohol **98** in 86% yield. The next step involves the preparation of the corresponding deprotected benzylic bromide **99** using HBr (g). Since a basic work up resulted in hydrolysis to the diol **100**,

Scheme 27: Target compounds **92**, **93**, and **94**, their oxidation products, and possible Diels-Alder adducts



92 n = 1 **95** n = 1

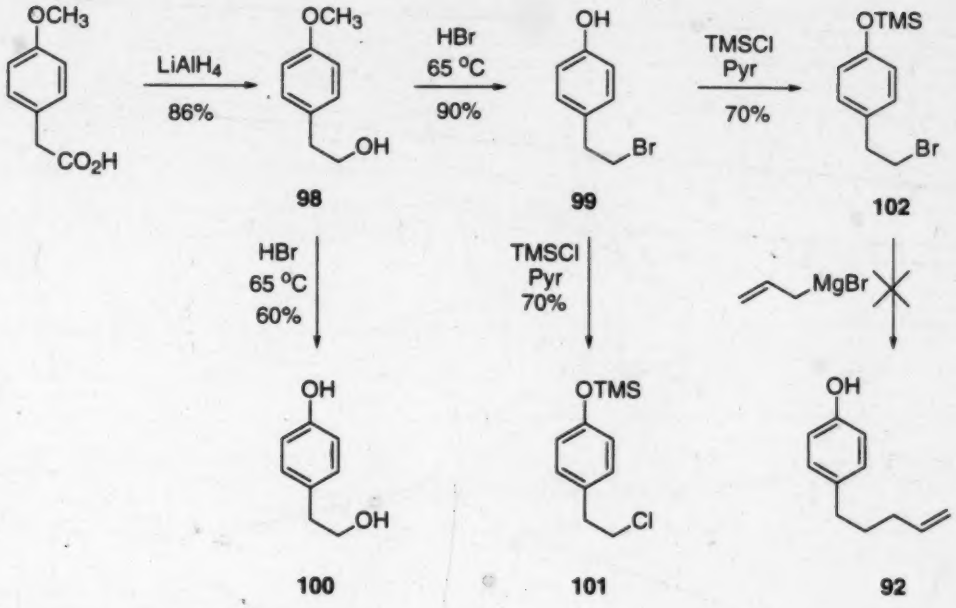
93 n = 2 **96** n = 2

94 n = 3 **97** n = 3

the reaction mixture is best partitioned between water and ether to afford compound **99** in 90% yield. Protection of the phenol proceeds readily with trimethylsilyl chloride and pyridine in THF. An acidic work up leads to the exchange of the bromide for a chloride from hydrochloric acid to give the protected compound **101** in 70% yield. In the absence of HCl, the desired bromide **102** is produced. The last step involves the formation of the Grignard reagent made from **102** and its attack on allyl bromide. As this reaction generates numerous unwanted products, alternative synthetic schemes were examined.

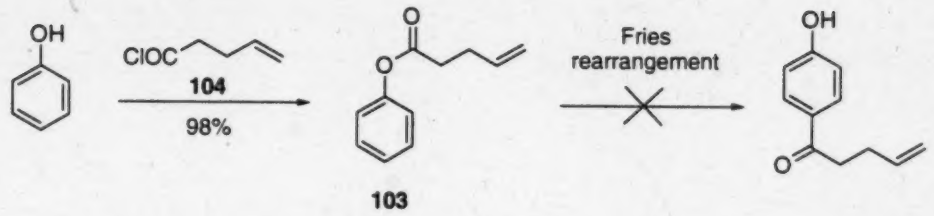
The Friedel-Crafts[146] alkylation of aromatic rings is a versatile reaction, but because the alkylated ring is generally more reactive than the starting material, it is often difficult to prevent multiple alkylations. However, the Friedel-Crafts acylation usually stops after one group is introduced. In both cases, phenol groups have to be protected to avoid *O*-alkylation/acylation when halides and acid halides are used. The related Fries rearrangement[147] involves heating phenolic esters with Friedel-Crafts catalysts to produce

Scheme 28: Approaches to synthesis of compound **92**



ortho- and/or *para*-acylphenols depending on the conditions used (temperature, solvent, and catalyst). Low temperatures generally favor the *para* product and high temperatures the *ortho*. We investigated the Fries rearrangement of the phenolic ester **103** to generate the *para*-acylated phenol, whose carbonyl could potentially be reduced under Clemmensen[148] conditions or via desulfurization[149] of its cyclic dithioketal. Compound **103** forms in 98% yield by heating the acid chloride **104** with phenol (Scheme 29). Unfortunately, the Fries rearrangement of this in presence of aluminum chloride generates a mixture of unreacted phenol and polymerized product.

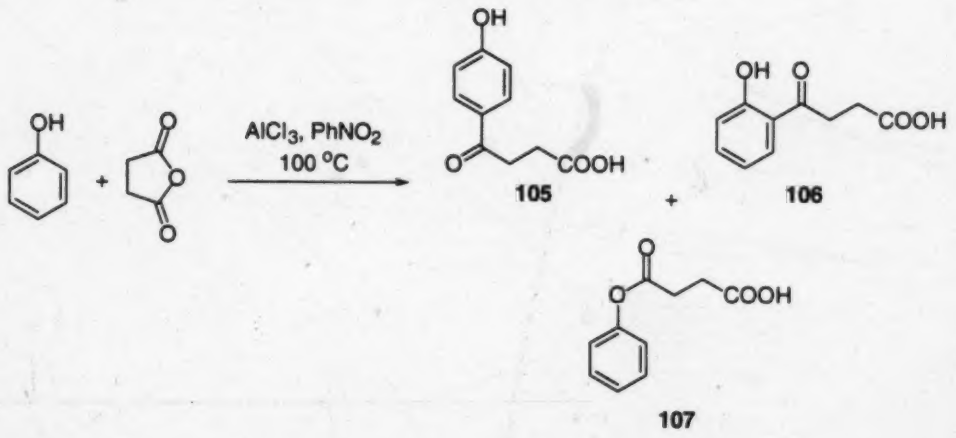
Scheme 29: Attempted Fries rearrangement of **103**



103

Another approach involves the use of a Friedel-Crafts reaction with an anhydride (Scheme 30). Thus, when phenol is treated with succinic anhydride and aluminum chloride, the desired *para*-phenol **105**, the *ortho*-compound **106**, and the *O*-acylated **107**, are obtained in low yield and in variable ratios. Attempted optimization of the conditions led to a maximum yield of 10% of compound **105**.

Scheme 30: Alkylation of phenol with succinic anhydride

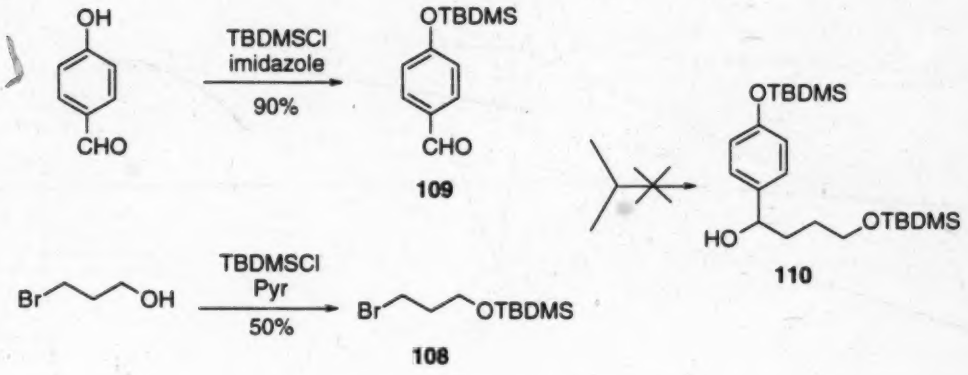


105 + **106**

107

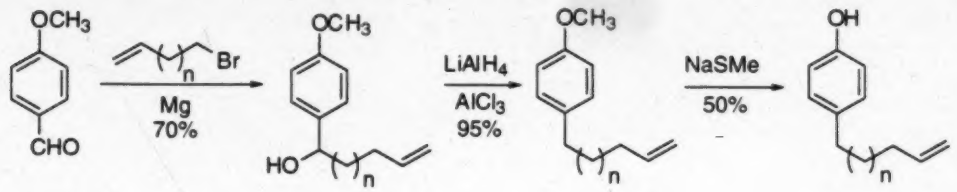
An alternative approach involves attachment of the side chain via Grignard reaction.[150] Protection of 3-bromopropanol as the *tert*-butyldimethylsilyl ether **108** proceeds readily as does the corresponding reaction on 4-hydroxybenzaldehyde to give **109** (Scheme 31). However, preparation of the magnesium or lithium reagents derived from **108** failed.

Scheme 31: Attempted formation of **110** via organometallic reactions



Commercially available 4-bromo-1-butene (**111**), 5-bromo-1-pentene (**112**), and 6-bromo-1-hexene (**113**) are suitable building blocks for the side chain of target compounds **92**, **93**, and **94**, respectively. Thus, reaction of 4-methoxybenzaldehyde with the Grignard reagents derived from **111**, **112**, or **113** affords the corresponding alcohols **114**, **115**, and **116**, respectively (Scheme 32). Reduction of the hydroxyl group using aluminum hydride prepared *in situ* from aluminum chloride and lithium aluminum hydride[151] proceeds with excellent yields to give **117**, **118**, and **119**, respectively. Deprotection to the target phenols **92**, **93**, and **94** is achieved with sodium thiomethoxide. Alternative methods for methyl ether cleavage using thiophenol,[152] trimethylsilyl iodide, and boron tribromide fail.

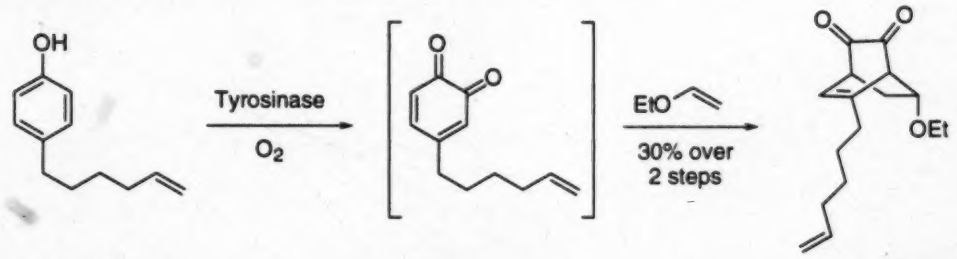
Scheme 32: Synthesis of target compounds **92**, **93**, and **94**



111 n = 1	**114** n = 1	**117** n = 1	**92** n = 1
112 n = 2	**115** n = 2	**118** n = 2	**93** n = 2
113 n = 3	**116** n = 3	**119** n = 3	**94** n = 3

The tyrosinase oxidation and Diels-Alder reactions of compounds **92**, **93**, and **94** were tested in the same way as for the *ortho*-phenol **82**. Their slow oxidation affords the unstable *ortho*-quinones **95**, **96**, and **97**, respectively, which quickly polymerize. In order to trap these transient molecules, the electron rich dienophile, ethyl vinyl ether, was added to enzymatic reactions. The *ortho*-quinone **96** could be successfully trapped into the racemic *exo* compound **120** (Scheme 33), but **95** and **97** polymerized before being captured in detectable amounts.

Scheme 33: Trapping of the *ortho*-quinone **96** with ethyl vinyl ether

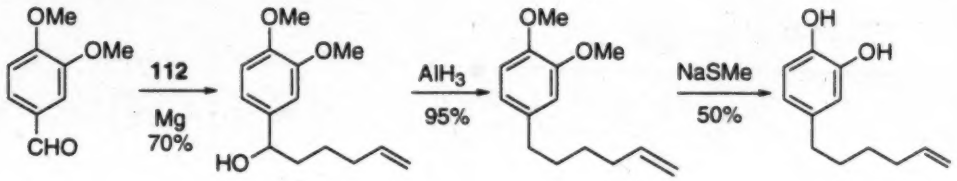


93 **96** **120**

Catechols for chemical oxidation to *ortho*-quinones. In order to examine the reactivity of *ortho*-quinones towards intramolecular Diels-Alder cyclization, chemical oxidation was also investigated. Compound **121** is available from 3,4-methoxybenzaldehyde using the same strategy as for **92**, **93**, and **94** (Scheme 34). The

Grignard reagent prepared from **112** reacts with 3,4-methoxybenzaldehyde to afford the alcohol **122**. Reduction with aluminum hydride gives compound **123**, which is deprotected with sodium thiomethoxide to the catechol **121**.

Scheme 34: Synthesis of compound **121**



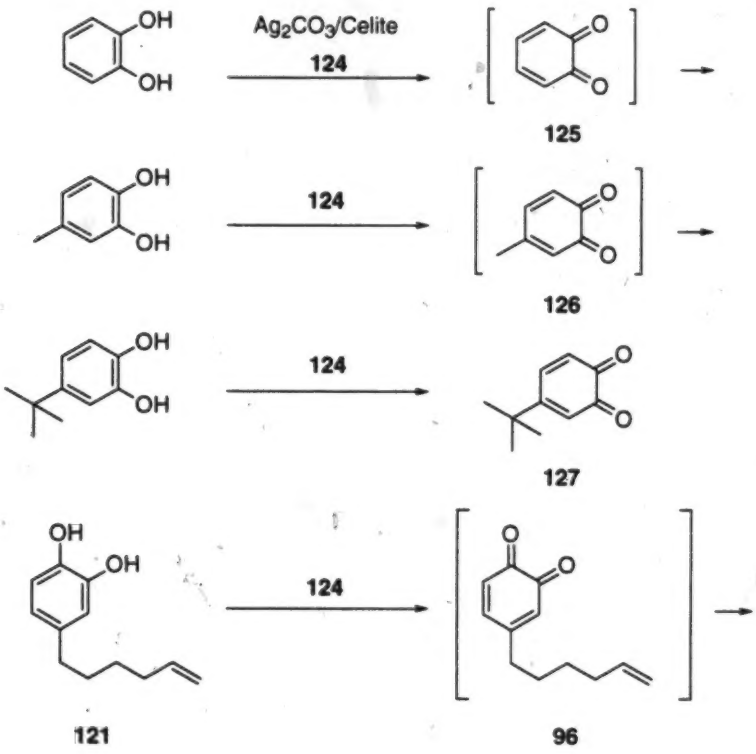
122 **123** **121**

Many reagents have been used for the oxidation of catechols to *ortho*-quinones, including Fremy's salt (potassium nitrosodisulfonate),[153] Fétizon reagent (silver carbonate on Celite (**124**)),[154] and diphenylseleninic anhydride.[155] *ortho*-Quinones,[156] are very reactive molecules, susceptible to both electrophilic and nucleophilic attack. They are also photolabile species that easily undergo polymerization and reduction.[157] Quinoids have been extensively tested in both normal and inverse electron demand Diels-Alder reactions.[156]

Silver carbonate on Celite (**124**) is prepared by the reaction of silver nitrate with potassium hydrogen carbonate in presence of Celite.[154] Its efficacy was first tested by oxidation of catechol, 4-methylcatechol, and 4-*tert*-butylcatechol (Scheme 35). *ortho*-Benzoquinone (**125**) is very unstable and undergoes rapid dimerization through a Diels-Alder reaction[157] as well as polymerization to give a mixture that is difficult to purify. The known 4-methyl-1,2-quinone (**126**)[158] also polymerizes quickly. However, the more hindered 4-*tert*-butyl-1,2-quinone (**127**)[158] is stable enough to allow its characterization. Trapping of these three quinones via Diels-Alder reactions with ethyl vinyl ether, 1,3-cyclohexadiene, or 1-pentyne was not successful and detectable amounts of characterizable adducts were not obtained. Not surprisingly, treatment of compound **121**

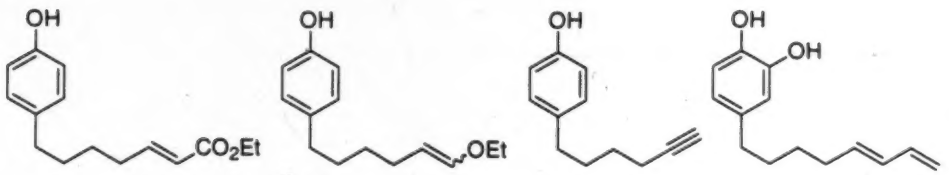
with silver carbonate on Celite (**124**) in dichloromethane also results in complex polymeric products.

Scheme 35: Chemical oxidation of catechols



Catechols for normal electron demand Diels-Alder reactions. Since the enzymatic oxidation of alkenyl phenols **92, 93,** and **94** is slow and the unsubstituted olefins did not undergo intramolecular Diels-Alder reaction, the preparation of compounds with electron rich or poor dienophiles, acetylene, or a diene was envisioned. The preparation of target compounds **128**, **129**, **130**, and **131** (Figure 38) began with compound **118** or **123** as before (Scheme 36). Treatment of **118** with osmium tetroxide

Figure 38: Target compounds **128**, **129**, **130**, and **131**



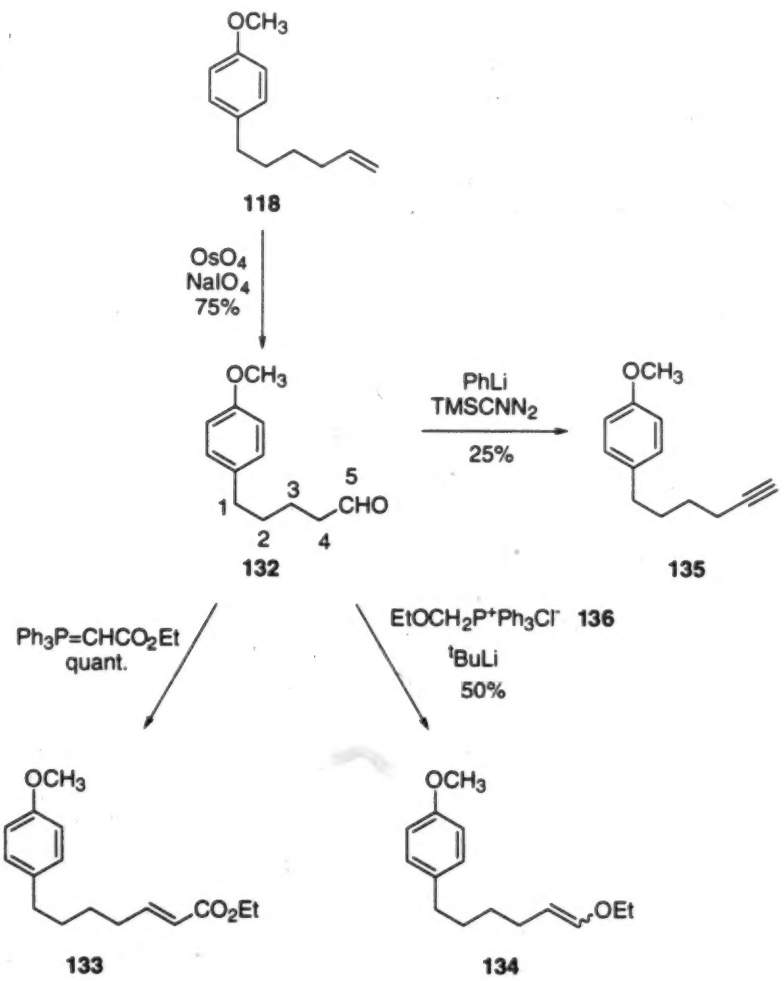
128 **129** **130** **131**

and sodium periodate under the conditions of Lemieux-Johnson oxidation,[159] affords the aldehyde **132** in 75 % yield (Scheme 36). This aldehyde is the branching point of the planned syntheses involving four different Wittig reactions.[160] Compound **132** reacts with the stabilized Wittig reagent in benzene to give the α,β-unsaturated ester **133**.

The aldehyde **132** condenses with **136**, prepared from triphenylphosphine and chloromethyl ethyl ether, to afford **134** as a mixture of *trans:cis* isomers in a 60:40 ratio, respectively. The mixture slowly hydrolyses to the corresponding aldehyde. According to ^{1}H NMR studies the *cis* isomer seems to hydrolyse faster. Isomerization experiments[161] using iodine followed by ^{1}H NMR spectroscopy in CD_2Cl_2 showed addition of iodine to the double bond, as well as hydrolysis. Isomerization at 25°C for two weeks does not go to completion (max 90% *trans*), and competing hydrolysis occurs. Synthesis of compound **129** was abandoned as reactivity tests in $CDCl_3$ showed that phenol and ethyl vinyl ether react to give the corresponding acetal, and also because facile hydrolysis of aryl and vinyl acetals is well known.[162]

Homologation of the aldehyde **132** to the alkyne **135** is readily achieved in 25% yield by treatment with a mixture of trimethylsilyl diazomethane via an application of a standard acetylene synthesis.[163] Attempts to remove the protecting group using sodium thiomethoxide in DMF at 100 °C were not successful. Surprisingly, the organic extract of the reaction contained either mostly unreacted **135** or an unstable mixture of non-aromatic compounds.

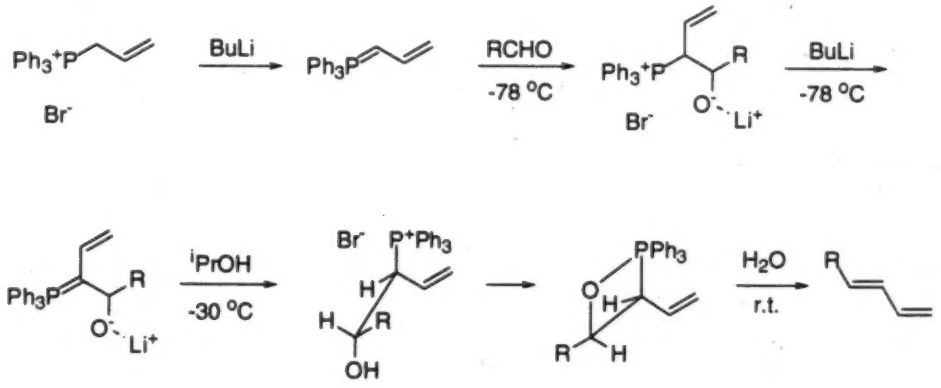
Scheme 36: Synthesis of compounds **133**, **134**, and **135**



In the Wittig reaction,[160] ylides with stabilizing groups or derived from trialkylphosphines generally give *E*-olefins, whereas other ylides predominantly produce

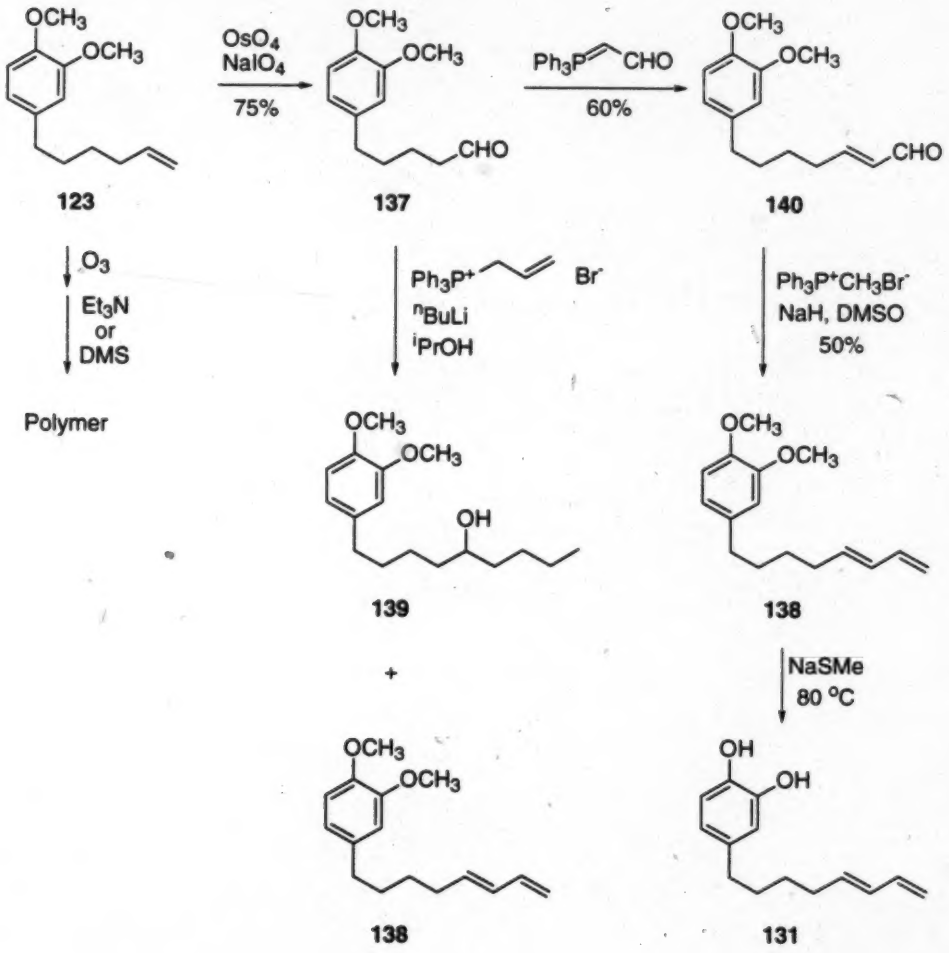
Z-olefins. In the Schlosser modification,[164] a strong base (BuLi) deprotonates the betaine-lithium bromide adduct and subsequent addition of a proton source (^{i}PrOH) generates the more stable *threo*-betaine-like adduct (Scheme 37). This variant therefore leads to predominantly *E*-olefins from non-stabilized ylides, and seemed ideal for preparation of diene systems such as **131** (Scheme 38).

Scheme 37: Mechanism of the Schlosser modification



Ozonolysis[165] of the terminal double bond of **123** gives a mixture of polymers, but osmium tetroxide and sodium periodate under the conditions of Lemieux-Johnson oxidation,[159] afford the aldehyde **137** in 75 % yield. The Schlosser modification of the Wittig reaction forms the diene **138** from the aldehyde **137** and allyl triphenylphosphonium bromide (Scheme 38). The unoptimized reaction generates the desired compound **138** in 10% yield, and the butyl adduct **139** in 55% yield.

Scheme 38: Synthesis of compound **131**

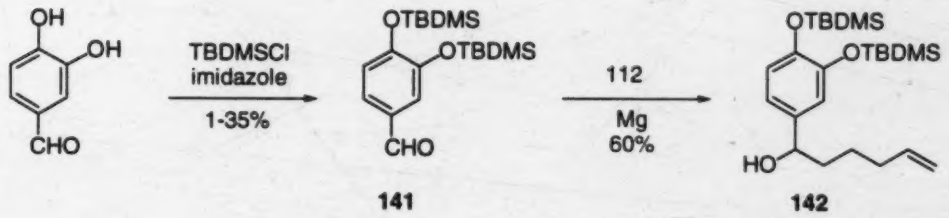


An alternative route to **138** using two consecutive Wittig reactions has an overall yield of 30%. Reaction of the aldehyde **137** with formylmethylenetriphenylphosphorane gives the α,β-unsaturated aldehyde **140**. Transformation of **140** with methyltriphenylphosphonium bromide in presence of sodium hydride in DMSO generates **138**. Deprotection of **138** by treatment with sodium thiomethoxide is very slow and

affords a mixture of mono-protected and fully deprotected compounds. Unfortunately, catechol **131** is unstable and decomposes during purification, so a completely homogeneous sample was not obtained.

Since the deprotection of methyl ethers requires harsh conditions, the di-*tert*-butyldimethylsilyl ether of 3,4-dihydroxybenzaldehyde **141** was prepared in 35% yield (Scheme 39). Treatment of **141** with the Grignard reagent prepared from 5-bromo-1-pentene (**112**) yields the alcohol **142**. Unfortunately, the protection reaction leading to the first intermediate **141** gives very low yields (1%) after attempted scale-up (five fold). At this stage further attempts to generate precursors for the enzyme-mediated oxidation to *ortho*-quinones with subsequent Diels-Alder condensation were abandoned. The limited scope combined with the general instability of the required precursors seemed to indicate that this particular approach was too cumbersome to be of general use and interest.

Scheme 39: The use of TBDMS protecting groups



141 **142**

5.9 Significance

The biosynthetic pathway to 4a,5-dihydromonacolin L (**11**) by the PKS was proposed to involve a biological Diels-Alder reaction at the hexaketide stage. When the triene **16**, which mimics the proposed hexaketide, was reacted with purified LNKS, the

enzyme was shown to catalyze the Diels-Alder reaction with the correct stereochemistry required for the biosynthesis of lovastatin (**1**). Many secondary metabolites may be produced *via* an enzymatic Diels-Alder reaction, but until now, this had experimentally been shown only for the biosynthesis of solanapyrones,[90] although that enzyme was never purified to homogeneity. LNKS is the first fully purified Diels-Alderase. These two enzymes are very different. Solanapyrone synthase is a normal size oxidase believed to catalyze two steps, whereas LNKS is a large multifunctional enzyme (335 kDa) with at least six active sites, each catalyzing more than one step.

LNKS was also tested for catalysis of Diels-Alder cyclization of trienes **59** and **60**, but did not prove effective. Model studies to verify the generality of enzyme-catalyzed Diels-Alder reactions were undertaken using the commercially available enzymes HLADH, BYADH, subtilisin Carlsberg, and carbonic anhydrase. For this purpose, a series of trienes were prepared by the tethering of pertinent dienes with dienophiles. None of the enzymatic reactions using these enzymes led to intramolecular Diels-Alder reaction.

Trienes were also generated *in situ* by the action of tyrosinase on various substrates. *ortho*- and *para*-Phenols were prepared with side chains of different length and a terminal double bond. The *ortho*-compounds were not accepted by the enzyme. The *para*-compounds were transformed to *ortho*-quinones that polymerized before intramolecular Diels-Alder reaction could occur. One of the quinones was trapped by intermolecular [4+2] cycloaddition giving a racemic product. Syntheses of phenols with more active unsaturated side chains were not successful.

EXPERIMENTAL

1. General

All non-aqueous reactions requiring anhydrous conditions were performed under a positive pressure of dry argon (Ar) using oven-dried glassware. All solvents for anhydrous reactions were dried according to Perrin *et al.*[166] THF, ether, benzene, and toluene were distilled over sodium and benzophenone under an Ar atmosphere. Acetonitrile, dichloromethane, DMF, DMSO, triethylamine and pyridine were distilled from calcium hydride. Anhydrous ethanol and methanol were purified by distillation from magnesium turnings and a catalytic amount of iodine. Water was obtained from a Milli-Q reagent water system (Millipore Corp.; Milford, MA). The removal of solvents was performed under reduced pressure using a Büchi rotary evaporator. "Brine" refers to a saturated solution of sodium chloride. Unless otherwise specified, solutions of ammonium chloride, sodium hydrogen carbonate, potassium hydrogen carbonate, sodium carbonate, lithium hydroxide, potassium hydroxide and sodium hydroxide refer to aqueous solutions.

Air sensitive reagents were handled under an atmosphere of dry Ar. All reagents employed were of the American Chemical Society (ACS) grade or finer. All commercially available labeled compounds were purchased from Cambridge Isotope Laboratories (Woburn, MA). Commercial organometallic reagents were obtained from Sigma-Aldrich Canada (Oakville, ON). Radioactive compounds were obtained from DuPont Canada (Mississauga, ON), or from Sigma-Aldrich Canada. All biological compounds/mixtures were obtained from Sigma-Aldrich Canada, from BDH Inc. (Toronto, Canada), or from Difco Laboratories (Detroit, MI), except for the Pablum (Meadjohnson ground mixed oral pablum) and the tomato paste (Hunt's) which were

obtained from the grocery store (Safeway Canada). The enzymes were purchased either from Sigma-Aldrich or from FLUKA (Oakville, ON). Electrophoresis gels and solutions were obtained from BioRad Canada (Mississauga, ON). All solutions, glassware and tools used for fermentation were autoclaved at 121°C and 30 psi.

Where possible all reactions were followed by thin layer chromatography (TLC) and visualized using UV fluorescence, iodine staining and/or phosphomolybdic acid:ceric sulfate:sulfuric acid (10 g:1.25g:12 mL in 240 mL of water) spray. Commercial thin layer chromatography plates were normal silica (Merck 60 F254) or reverse phase (Merck RP-8 or RP-18 F254S) and 5 cm in length. Flash column chromatography was performed according to Still *et al.*[167] using Merck type 60, 230-420 mesh silica gel or its equivalent from General Intermediates of Canada (Edmonton, AB). All solvent mixtures are listed as volume ratios.

High pressure liquid chromatography (HPLC) was performed either on a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector, and an Altex 210A injector, or on a Rainnin Dynamax instrument equipped with a variable wavelength model UV-1 detector, a solvent delivery system model SD-200, and a Rheodyne injector. The columns were Novapak, Resolve, μBondapak, or μPorasil cartridges encased in a Waters Z-module compression unit. HPLC grade acetonitrile (190 nm UV cutoff) and methanol were obtained from Fisher (Fair Lawn, NJ). All HPLC solvents were filtered daily with a Millipore vacuum filtration system before use.

All literature compounds had IR, ^{1}H NMR and mass spectra consistent with the assigned structure. Melting points are uncorrected and were determined on a Kauffmann Block microscope, or on a Thomas-Hoover apparatus using open capillary tubes. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (100 mm path length, 1 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line and were referenced against air. Infrared

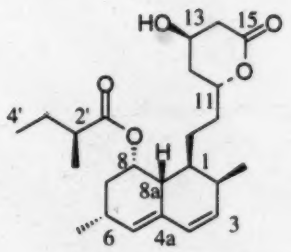
spectra (IR) were recorded on a Nicolet Magna-IR 750 with Nic-Plan Microscope FT-IR spectrometer. Cast refers to the evaporation of a solution on a sodium chloride plate. The abundance of each ion in the mass spectra (MS) is reported in percentage. MS were recorded on a Kratos AEI MS-50 (high resolution, electron impact ionization (EI)), a VG-70 (low resolution, chemical ionization (CI)), a HP 1100 (low resolution, electron spray ionization (ES)), and an ZabSpec Iso Mass VG (high resolution, ES). Microanalyses were obtained using Carlo Erba 1108 CHN analyzer.

Radioactivity was determined using standard liquid scintillation procedures in plastic 10 mL scintillation vials (Terochem) with Beckman Ready gel scintillation cocktail (Fullerton, CA). The instrument used was a Beckman LS 5000TD with automatic quench control to directly determine decompositions per minute (dpm) in the labeled samples by comparison against a quench curve prepared from Beckman ^{3}H and ^{14}C quenched standards.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker AM-300, WM-360, WH-400, or on Varian Unity 500 or Inova 600 instruments. ^{1}H NMR chemical shifts for the Brukers are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using solvent resonance as the reference: $CDCl_3$ δ 7.24, CD_2Cl_2 δ 5.32, CD_3OD δ 3.30, $(CD_3)_2CO$ δ 2.04, CD_3CN δ 1.93. ^{13}C NMR shifts are reported relative to $CDCl_3$ δ 77.00, CD_2Cl_2 δ 53.80, CD_3OD δ 49.00, $(CD_3)_2CO$ δ 29.80, CD_3CN δ 117.70. ^{1}H and ^{13}C NMR chemical shifts for the Varians are reported in parts per million (ppm) using external 10% acetone reference. Attached proton test (APT), distortionless enhancement by polarisation transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), or correlated spectroscopy (COSY) were occasionally used for signal assignments. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; p, pentet (quintet); m, multiplet), number of protons, coupling constant(s) in Hertz (Hz), and assignment.

The ß-oxidation inhibitor ethyl 3-hydroxypent-4-ynoate (**26**) and *N*-acetylcysteamine (NAC) were synthesized using Dr. Zhe Li's thesis procedure.[168] The latter was also purchased from Sigma-Aldrich Canada. The hexaketides **16** and **16a** were synthesized by Mr. Johan P. van den Heever as previously reported by our group.[46] Compound **28a** was prepared by Dr. Yaoquan Liu as described elsewhere.[5,97] Compound **57** was prepared by David J. Witter in our group as reported before.[46]

2. Experimental Data for Compounds



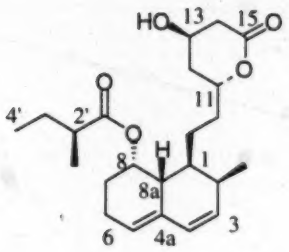
Lovastatin (1). Lovastatin (**1**) was isolated from *A. terreus* control, *lovE*, and XS-1P, fermented as described in the fermentation section or below. Compound **1** was also isolated after incorporation studies of monacolin J (**14**) and 4a,5-dihydromonacolin L (**11**) using *A. terreus lovC*, and after incorporation studies of [1,2-$^{13}C_2$]-acetyl CoA (**39a**) using 'protoplasts' of *A. terreus* XS-1P, by the methods described with the fermentation procedures.

Lovastatin was isolated from *A. terreus lovE* and control as follows. The grown cultures (1 L) were blended for 30 sec. After acidification to pH 3, ethyl acetate was added (500 mL), the mixture was blended for 1 min twice, and vacuum filtered through

two filter papers. The solid residue collected was washed with ethyl acetate (100 mL). The aqueous layer of the filtrate was saturated with NaCl and extracted with ethyl acetate (3 × 200 mL). Combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. In order to ensure that all the lovastatin (**1**) was lactonized, toluene (60 mL) was added to the residue (ca. 1 g) and the solution was heated under reflux with a sohxlet containing calcium hydride for 90 min. The toluene was removed *in vacuo* and the lovastatin (**1**) was quantified by analytical HPLC. The column used was a μBondapak radial compression reverse phase C_8, 8 × 100 mm, 4 μm (Millipore Waters, Mississauga, ON). The flow rate was 2 mL/min, and the gradient was kept at 40% acetonitrile in water and 0.1% TFA for 5 min, then raised from 40% to 52% over 20 min, kept at 52% for 10 min, raised from 52% to 95% over 5 min, and kept at 95% for 10 min. The UV detector was set at 240 nm. Retention time was 21 min for lovastatin (**1**). The *A. terreus lovE* strain was found to produce approximately three times more lovastatin (**1**) than the control strain. Lovastatin (**1**) was purified by HPLC with a μBondapak radial compression reverse phase C_{18}, 40 × 100 mm, 10 μm, 300 Å column (Millipore Waters, Mississauga, ON). The flow rate was kept at 32 mL/min, and the gradient was raised from 45% acetonitrile in water and 0.1% TFA, to 50% over 8 min, raised from 50% to 63% over 15 min, from 63% to 100% over 2 min, and kept at 100% for 5 min. The UV detector was set at 240 nm. Retention time was 16 min for lovastatin (**1**). Compound **1** was obtained as a white solid (ca. 100 mg/L of cultures).

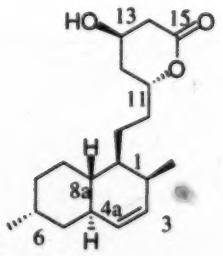
Lovastatin (**1**) was also isolated from *A. terreus* XS-1P as follows. The cultures (1 L) were vacuum filtered through two filter papers. The filtrate was extracted with ethyl acetate (3 × 250 mL) after acidification to pH 3 (2N HCl) and saturation with NaCl. The wet mycelia (60 g) were blended in water (100 mL) for 30 sec at low speed, then for an additional 1 min at low speed after addition of ethyl acetate (150 mL). The suspension was vacuum filtered through a bed of Celite. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. HPLC was used for monitoring

lovastatin (**1**) production and to search for new metabolites. The column used was a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was kept at 40% acetonitrile in water and 0.1% TFA for 10 min, then raised from 40% to 49% over 11 min, kept at 49% for 8 min, raised from 49% to 56% over 8 min, from 56% to 95% over 2 min, and kept at 95% for 9 min. The UV detector was set at 215 nm or 220 nm to allow detection of 4a,5-dihydromonacolin L (**11**). Retention time was 32 min for lovastatin (**1**). The *A. terreus* XS-1P cultures were compared against the ancymidol inhibited cultures and the wild strain. The lovastatin (**1**) production was found to be in a ratio of 30:10:1 respectively while the wet mycelia production ratio was 2.0:1.4:1.0 respectively. $[\alpha]_D^{25}$ = +314 (*c* 1.8, CH_3CN), lit. $[\alpha]_D^{25}$ = +323 (*c* 0.84, CH_3CN);[31a] UV (MeOH solution) λmax 238, 204 nm; IR ($CHCl_3$ cast) 3435 (br m), 2963 (m), 1725 (s), 1258 (m), 1187 (m) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.94 (d, 1H, *J* = 9.7 Hz, H-4), 5.74 (dd, 1H, *J* = 9.7, 6.0 Hz, H-3), 5.47 (m, 1H, H-5), 5.34 (ddd, 1H, *J* = 3.1, 3.0, 3.1 Hz, H-8), 4.59 (m, 1H, H-11), 4.31 (m, 1H, H-13), 3.29 (br s, 1H, OH), 2.67 (dd, 1H, *J* = 17.7, 5.0 Hz, H_a-14), 2.59 (dd, 1H, *J* = 17.7, 2.4 Hz, H_b-14), 2.40 (m, 1H, H-6), 2.36-2.27 (m, 2H, H-2, H-2'), 2.23 (dd, 1H, *J* = 12.0, 3.1 Hz, H-8a), 1.96-1.77 (m, 4H, H-7, H_a-10, H_a-12), 1.68-1.57 (m, 3H, H-1, H_a-3', H_b-12), 1.49-1.30 (m, 3H, H-9, H_b-3'), 1.25 (m, 1H, H_b-10), 1.06 (d, 3H, *J* = 7.0 Hz, 2'-Me), 1.03 (d, 3H, *J* = 7.3 Hz, 6-Me), 0.85 (d, 3H, *J* = 7.0 Hz, 2-Me), 0.84 (t, 3H, *J* = 7.5 Hz, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.95 (C-1'), 170.78 (C-15), 132.99 (C-3), 131.50 (C-4a), 129.53 (C-5), 128.24 (C-4), 76.46 (C-11), 67.93 (C-8), 62.38 (C-13), 41.45 (C-2'), 38.51 (C-14), 37.23 (C-8a), 36.54 (C-1), 35.98 (C-12), 32.89 (C-10), 32.62 (C-7), 30.61 (C-2), 27.37 (C-6), 26.74 (C-3'), 24.18 (C-9), 22.76 (6-Me), 16.16 (2'-Me), 13.80 (2-Me), 11.65 (C-4'); MS (ES) calcd for $C_{24}H_{37}O_5$ 405.2641, found 405.2638 (MH^+).



Compactin (5). The original isolation of compactin (**5**) was from *Penicillium aurantiogriseum* cultures (1 L) fermented as described in the fermentation section. Compactin (**5**) was also isolated after incorporation studies with compounds **28a** and **29a** using *P. aurantiogriseum*, and compound **52** using the *A. terreus lovC* strain, but not after incorporation studies of ML-236C (**25**) using the *A. terreus lovC* mutant (see fermentation section). Compactin (**5**) was initially isolated from *P. aurantiogriseum* as follows. The cultures were vacuum filtered through two filter papers. The filtrate was acidified to pH 2 (2N HCl), saturated with NaCl, and extracted with ethyl acetate (4 × 150 mL). The mycelia were blended with water (150 mL) at low speed for 30 sec, acidified to pH 2 (2N HCl), then blended with ethyl acetate (400 mL) for 1 min. The mixture was then filtered through a bed of Celite. The filtrate was further extracted with ethyl acetate (300 mL) and all the combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. In order to ensure that all the compactin was lactonized, toluene (100 mL) was added to the residue (280 mg) and the solution was heated under reflux with a sohxlet containing calcium hydride for 90 min. The toluene was removed *in vacuo* and compactin (**5**) was pre-purified using silica gel column chromatography (30 to 70% ethyl acetate in hexanes, R_f 0.35 with 30%). Final purification of compactin (**5**) was achieved by HPLC using a μBondapak radial compression reverse phase C_8, 25 × 100 mm, 15-20 μm, 300 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min. The gradient was kept at 40% acetonitrile in water and 0.1% trifluoroacetic acid (TFA) for 10 min, then raised from 40% to 49% over 11 min, kept at

49% for 8 min, raised from 49% to 56% over 8 min, from 56% to 95% over 2 min, and kept at 95% for 9 min. The UV detector was set at 238 nm. The retention time was 37 min for compactin (**5**). Compound **5** was obtained as a white solid (100 mg/L). $[\alpha]_D^{25}$ = +158 (*c* 0.5, $CHCl_3$), lit. $[\alpha]_D^{25}$ = +283 (*c* 0.84, acetone);[31b] IR ($CHCl_3$ cast) 3407 (br w), 2925 (m), 1725 (s), 1251 (m), 1077 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.96 (d, 1H, *J* = 9.6 Hz, H-4), 5.72 (dd, 1H, *J* = 9.6, 6.0 Hz, H-3), 5.54 (br d, 1H, *J* = 2.6 Hz, H-5), 5.32 (br t, 1H, *J* = 4.3 Hz, H-8), 4.60 (m, 1H, H-11), 3.86 (m, 1H, H-13), 2.72 (dd, 1H, *J* = 17.6, 5.2 Hz, H_a-14), 2.60 (ddd, 1H, *J* = 17.6, 4.0, 1.5 Hz, H_b-14), 2.36 (m, 3H, H-2, H-6), 2.31 (m, 1H, H-8a), 2.13 (m, 4H, H-1, H-7, OH), 1.95 (m, 2H, H-12), 1.45-1.24 (m, 6H, H-3', H-9, H-10), 1.11 (d, 3H, *J* = 7.0 Hz, 2-Me), 0.88 (m, 6H, 2-Me, 4'-Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.89 (C-1'), 170.49 (C-15), 133.66 (C-4a), 132.75 (C-3), 128.26 (C-4), 123.78 (C-5), 76.34 (C-11), 67.67 (C-8), 62.70 (C-13), 41.81 (C-6), 38.67 (C-2'), 37.62 (C-14), 36.94 (C-8a), 36.26 (C-12), 33.06 (C-10), 30.96 (C-2), 26.81 (C-3'), 26.34 (C-7), 24.11 (C-9), 21.02 (C-1), 17.00 (2'-Me), 13.94 (2-Me), 11.85 (4'-Me); MS (EI) calcd for $C_{23}H_{34}O_5$ 390.2406, found 390.2409 (M^+, 5), 270.1620 (M-$C_5H_{12}O_3$, 8), 210.1402 ($C_{16}H_{18}^+$, 16), 184.1251 ($C_{14}H_{16}^+$, 60), 145.1018 ($C_{11}H_{13}^+$, 87), 57.0697 ($C_4H_9^+$, 100).



4a,5-Dihydromonacolin L (11). 4a,5-Dihydromonacolin L (**11**) was isolated from many different experiments: incorporation studies of compound **29a**, of sodium

acetate, and of diethyl malonate using the *A. nidulans lovB+lovC* strain (described in fermentation section), as well as with cell free extracts from the same fungi which were fed with compounds **28a**, **29a**, and **16a** (described in cell free extracts section).

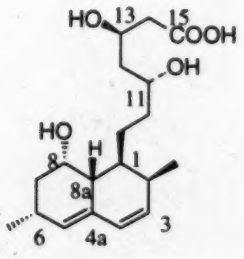
4a,5-Dihydromonacolin L (**11**) was originally isolated from the *A. nidulans lovB+lovC* strain fermented as described in the fermentation section. The culture (1 L in a 2 L Erlenmeyer) was vacuum filtered, and the acidified filtrate (2N HCl, pH 2) was extracted with ethyl acetate (3 × 300 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. In order to ensure that all the 4a,5-dihydromonacolin L (**11**) was lactonized, toluene (25 mL) was added to the residue (110 mg) and the solution was heated under reflux with a sohxlet containing calcium hydride for one hour. The toluene was removed *in vacuo* and the compound was pre-purified by HPLC on a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 300 Å column (Millipore Waters, Mississauga, ON). The flow rate was kept at 15 mL/min, and the gradient was raised from 30% acetonitrile in water and 0.1% TFA, to 40% over 10 min, raised from 40% to 49% over 11 min, kept at 49% for 6 min, then raised from 49% to 100% over 3 min, and kept at 100% for 7 min. The UV detector was set at 200 nm. Retention time was 21 min for 4a,5-dihydromonacolin L (**11**). The compound was further purified by recrystallization from ethanol/water, affording white needles (~20 mg/L of culture). Mp 162-163 °C, lit mp 163-164 °C;[44d] $[\alpha]_D^{25}$ = +115 (*c* 0.22, CH_3OH), lit. $[\alpha]_D^{25}$ = +123.9 (*c* 0.5, CH_3OH); UV (MeCN solution) λmax 228, 210-234 (br); IR (microscope) 3391 (br m), 3018 (w), 2910 (s), 1713 (s), 1254 (s), 1062 (s), 1047 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.57 (ddd, 1H, *J* = 9.8, 4.9, 2.7 Hz, H-3), 5.28 (d, 1H, *J* = 9.8 Hz, H-4), 4.67 (m, 1H, H-11), 4.38 (m, 1H, H-13), 2.72 (dd, 1H, *J* = 17.6, 5.1 Hz, H_a-14), 2.60 (ddd, 1H, *J* = 17.6, 3.7, 1.6 Hz, H_b-14), 2.21 (m, 1H, H-2), 2.00 (m, 1H, H-6), 1.95 (m, 1H, H_a-12), 1.89 (m, 1H, H-4a), 1.81-1.73 (m, 2H, H_a-10, H_b-12), 1.62 (m, 2H, H-9), 1.58-1.42 (m, 6H, H-5, H-7, H-8a, H_b-10), 1.07 (dq, 1H, *J* = 11.9, 3.7 Hz, H_a-8), 0.97 (dq, 1H, *J* = 11.9, 3.0 Hz, H_b-8), 0.96 (d, 3H, *J* = 7.3 Hz, 6-

Me), 0.82 (d, 3H, J = 6.9 Hz, 2-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.42 (C-15), 132.60 (C-3), 131.63 (C-4), 76.07 (C-11), 62.85 (C-13), 41.41 (C-8a), 39.97 (C-8), 38.92 (C-5), 38.66 (C-14), 37.28 (C-4a), 36.11 (C-12), 33.20 (C-10), 32.25 (C-7), 31.99 (C-2), 27.46 (C-6), 23.67 (C-1), 23.63 (C-9), 18.20 (6-Me), 14.95 (2-Me); MS (EI) calcd for $C_{19}H_{30}O_3$ 306.2195, found 306.2190 (M^+, 27), 288.2088 (M-H_2O, 18), 161.1330 (M-$C_7H_{13}O_3$, 100), 105.0704 ($C_8H_9^+$, 99).

Crystal data for 4a,5-dihydromonacolin L (11). Data were acquired on a Bruker P4/RA/SMART 1000 CCD diffractometer. All intensity measurements were performed using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). 4a,5-Dihydromonacolin L (**11**) ($C_{19}H_{30}O_3$) was obtained as white crystals, space group $P2_12_12_1$ (No. 19),[169] a = 5.5947 (9), b = 9.6504 (17), c = 31.930 (6) Å, V = 1723.9 (5) Å^3, Z = 4, T = -80 °C, ρ_{calcd} = 1.181 g cm^{-3}, μ = 0.078 mm^{-1}. A total of 10870 reflections were collected, of these, 3287 were unique. Due to the small crystal size and the very low value of the linear absorption coefficient, no absorption correction was performed. The structure was solved by direct methods (*SHELXS-86*),[170] and refined by full-matrix least-squares methods on F^2 (*SHELXL-93*).[171] In the final refinement cycle 3287 reflections with $F_o^2 \geq -3\sigma(F_o^2)$ were used and 200 parameters varied; the model converged with unweighted and weighted agreement factors R_1 = 0.0620 (for 899 data with $F_o^2 > 2\sigma(F_o^2)$) and wR_2 = 02159 (for all 3287 data), with a goodness-of-fit indicator (S) of 0.779. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

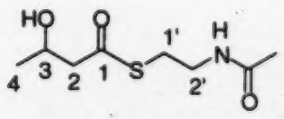
^{13}C-Labeled 4a,5-Dihydromonacolin L (11a) from incorporation of sodium [1,2-$^{13}C_2$]-acetate. Fermentation of the *A. nidulans lovB+lovC* strain with feeding of sodium [1,2-$^{13}C_2$]-acetate as described in the fermentation section, and purification as described for compound **11**, afforded **11a**. The experimental data for compound **11a** are the same as for compound **11** except for the following. ^{1}H NMR spectrum for

labeled compound in 20% labeled mixture (600 MHz, $CDCl_3$) δ 5.57 (br d, 1H, J_{CH} ~ 145 Hz, H-3), 5.28 (br d, 1H, J_{CH} ~ 156 Hz, H-4), 4.67 (br d, 1H, J_{CH} ~ 143 Hz, H-11), 4.38 (br d, 1H, J_{CH} ~ 153 Hz, H-13), 2.72 (br d, 1H, J_{CH} ~ 122 Hz, H-14), 2.60 (br d, 1H, J_{CH} ~ 133.0 Hz, H-14), 2.21 (br d, 1H, J_{CH} ~ 122 Hz, H-2), 2.05-1.40 (m, 13H, H-4a, H-5, H-6, H-7, H-8a, H-9, H-10, H-12), 1.40-1.00 (m, 2H, H-8), 0.82 (br d, 3H, J_{CH} ~ 125 Hz, 2-Me); ^{13}C NMR for 20% labeled compound (125 MHz, $CDCl_3$) δ 170.05 (br d, J_{CC} ~ 51 Hz, C-15), 132.60 (br d, J_{CC} ~ 60 Hz, C-3), 131.65 (br d, J_{CC} ~ 60 Hz, C-4), 76.05 (m, C-11), 62.85 (m, C-13), 41.40 (m, C-8a), 39.90 (m, C-8), 38.90 (m, C-5, C-14), 37.28 (m, C-4a), 36.11 (ddd, J_{CC} = 34.9, 15.9, 15.9 Hz, C-12), 33.20 (ddd, J_{CC} = 35.0, 16.5, 16.5 Hz, C-10), 32.00 (m, C-2, C-7), 27.46 (ddd, J_{CC} = 32.0, 14.0, 14.0 Hz, C-6), 23.50 (m, C-1, C-9), 18.20 (s, 6-Me), 14.95 (br d, J_{CC} ~ 34 Hz, 2-Me); MS (EI) calcd for $^{13}C_{18}C_1H_{30}O_3$ 324.2800, found 328.2789 (M^+, 2).

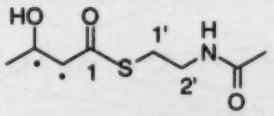


Monacolin J (14). A monacolin J (**14**) standard was prepared by Dr. Joanna Harris in our group by hydrolysis of lovastatin (**1**). Monacolin J (**14**) was detected in cultures of the *A. terreus* XS-1P strain treated with ancymidol (see fermentation section), and finally was purified from *A. terreus lovD* and *lovF* mutants grown as described in the fermentation section. Monacolin J (**14**) was isolated from the cultures as follows. The mycelia were filtered through two filter papers, and the filtrate was extracted with ethyl acetate (3 × 150 mL). The wet mycelia (30-45 g) were acidified to

pH 3 (2N HCl), blended for 30 sec in water (100 mL), and then for 1 min after addition of ethyl acetate (200 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was evaporated *in vacuo*. The residue was purified by HPLC with a μBondapak radial compression reverse phase column C_{18}, 25 × 100 mm, 10 μm, 125 Å (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min. The gradient started at 40% acetonitrile and 0.1% TFA for 10 min, raised from 40% to 49% over 11 min, kept at 49% for 8 min, raised from 49% to 56% over 8 min, from 56% to 100% over 5 min, and kept at 100% for 5 min. The UV detector was set at either 240 nm. Monacolin J (**14**) was collected as a white solid with a retention time of 10 min. $[\alpha]_D^{25}$ = +17.3 (*c* 0.14, CH_3OH); IR (microscope) 3236 (br s), 2927 (s), 2879 (s), 1707 (m), 1645 (m), 1451 (s), 1318 (s), 1093 (s), 1075 (s), 1060 (s), 1049 (s), 1026 (s), 973 (s), 858 (s) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.93 (d, 1H, *J* = 10.0 Hz, H-4), 5.75 (dd, 1H, *J* = 10.0, 6.2 Hz, H-3), 5.45 (br s, 1H, H-5), 4.23 (br dd, 1H, *J* = 6.5, 2.8 Hz, H-8), 3.92 (m, 1H, H-11), 3.79 (m, 1H, H-13), 3.68 (br t, 2H, *J* = 6.5 Hz, H-14), 2.38 (m, 2H, H-2, H-6), 2.13 (br dd, 1H, *J* = 12.1, 2.8 Hz, H-8a), 1.92-1.68 (m, 4H, H-7, H_a-9, H_a-12), 1.67-1.50 (m, 3H, H-1, H_a-10, H_b-12), 1.41 (m, 1H, H_b-10), 1.31 (m, 1H, H-9), 1.18 (d, 3H, *J* = 7.4 Hz, 6-Me), 0.89 (d, 3H, *J* = 6.9 Hz, 2-Me); ^{13}C NMR (125 MHz, CD_3OD) δ 189.96 (C-15), 134.11 (C-4), 133.16 (C-4a), 130.60 (C-3), 130.02 (C-5), 71.73 (C-8), 69.21 (C-11), 65.89 (C-13), 60.08 (C-14), 45.41 (C-7), 40.98 (C-10), 39.77 (C-8a), 37.61 (C-1), 37.10 (C-12), 35.64 (C-9), 32.13, 29.13 (C-2, C-6), 23.65 (6-Me), 14.29 (2-Me); MS (ES) 347 (MNa^+, 100), 671 ($2MNa^+$, 25).



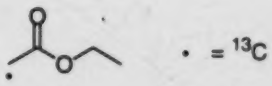
3-Hydroxybutyric acid, *N*-acetylcysteamine thioester (29). A modification of the procedure of Liu *et al.*[5] was used. To a solution of 3-(*tert*-butyldimethylsilyloxy)butyric acid, *N*-acetylcysteamine thioester **36** (0.52 g, 1.6 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added boron trifluoride etherate (1.5 mL, 12 mmol) dropwise. The mixture was stirred at 0 °C for 6.5 hours, then poured into 5% sodium hydrogen carbonate (125 mL) and extracted with $CHCl_3$ (4 × 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (100% ethyl acetate, R_f 0.12) to afford the product **29** as an oil (0.34 g, quant.). IR (neat) 3296 (br s), 3090 (m), 2971 (m), 1685 (s), 1658 (s), 1554 (s), 1375 (s), 1119 (s), 1005 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.25 (br s, 1H, NH), 4.22 (m, 1H, H-3), 3.40 (br q, 2H, J = 6.2 Hz, H-2'), 3.15 (br s, 1H, OH), 3.01 (m, 2H, H-1'), 2.68 (br d, 2H, J = 5.9 Hz, H-2), 1.93 (s, 3H, $C\underline{H}_3CON$), 1.21 (d, 3H, J = 6.2 Hz, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.13 (C-1), 170.68 ($CH_3\underline{C}ON$), 64.99 (C-3), 52.56 (C-2), 39.20 (C-1'), 28.77 (C-2'), 23.094 ($\underline{C}H_3CON$), 22.77 (C-4); MS (EI) calcd for $C_8H_{16}NO_3S$ 206.0851, found 206.0934 (MH^+, 1), 119.0406 (NAC^+, 100), 60.0451 ($C_2H_6NO^+$, 97); Anal. Calcd for $C_8H_{15}NO_3S$: C, 46.81; H, 7.37; N, 6.82. Found: C, 46.22; H, 7.59; N, 6.24.



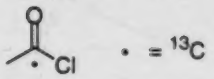
• = ^{13}C

[2,3-$^{13}C_2$]-3-Hydroxybutyric acid, *N*-acetylcysteamine thioester (29a). A modification of the procedure of Liu *et al.*[5] was used. To a solution of [2,3-$^{13}C_2$]-3-(*tert*-butyldimethylsilyloxy)butyric acid, *N*-acetylcysteamine thioester **36a** (0.248 g, 0.77 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added boron trifluoride diethyl etherate (0.7

mL, 5.6 mmol) dropwise. The mixture was stirred at 0 °C for 10 hours, then poured into 5% sodium hydrogen carbonate (60 mL) and extracted with $CHCl_3$ (4 × 50 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (100% ethyl acetate, R_f 0.12) to afford the alcohol **29a** as an oil (46.5 mg, 30%). IR (neat) 3296 (br s), 3090 (m), 2971 (m), 1685 (s), 1658 (s), 1554 (s), 1375 (s), 1119 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.30 (br s, 1H, NH), 4.20 (br d, 1H, J ~ 130.0 Hz, H-3), 3.35 (t, 2H, J = 6.2 Hz, H-2'), 3.15 (br s, 1H, OH), 3.00 (m, 2H, H-1'), 2.70 (dt, 2H, J = 128.8, 6.2 Hz, H-2), 1.90 (s, 3H, $C\underline{H}_3CO$), 1.20 (dt, 3H, J = 6.2, 4.7 Hz, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.12 (C-1), 170.73 ($\underline{C}ON$), 64.99 (d, J = 36.2 Hz, C-3), 52.57 (d, J = 36.2 Hz, C-2), 39.20 (C-1'), 28.75 (C-2'), 23.11 and 22.78 (C-4 and $\underline{C}H_3CO$), MS (EI) calcd for $C_8H_{16}NO_3S$ 206.0851, found 206.0850 (MH^+, 1), 119.0406 (M-NAC, 100), 60.0451 (H_3N^+Ac, 97).

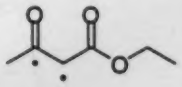


[2-^{13}C]-Ethyl acetate (30a). Triethyl phosphate (60 mL, 350 mmol) and sodium [2-^{13}C]-acetate (9.8 g, 118 mmol) were mixed and heated under reflux for 4 hours. The ester was distilled under a stream of Ar affording the product **30a** as a colorless liquid (8.1 g, 77%). Bp 76 °C; IR (neat) 2984 (s), 1740 (s), 1369 (s), 1354 (s), 1044 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.10 (q, 2H, J = 7.2 Hz, H-1'), 2.05 (d, 3H, J = 129.2 Hz, H-2), 1.25 (t, 3H, J = 7.2 Hz, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 217.68 (C-1), 60.36 (C-1'), 21.00 (C-2), 14.18 (C-2'); MS (EI) 88 (M^+, 29), 71 (M-H_2O, 47), 62 (M-Et, 62), 44 (M-OEt, 100).



• = ^{13}C

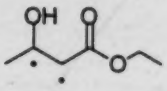
[1-^{13}C]-Acetyl chloride (31a). In a glove box and with vigorous stirring, phosphorous pentachloride (42.9 g, 206 mmol) was added to sodium [1-^{13}C]-acetate (10.6 g, 128 mmol) over 40 min. The mixture was heated under reflux for three hours. The reaction mixture was distilled under a stream of Ar affording **31a** as a colorless liquid (13.6 g, quant.). Bp 62 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.66 (d, 3H, J = 7.6 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.16 (C-1), 68.55 (C-2).



• = ^{13}C

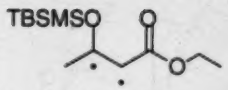
Ethyl [2,3-$^{13}C_2$]-acetoacetate (32a). A modification of the procedure of Block and Cane[172] was used. LiHMDS was prepared by the addition of *n*-BuLi (40 mL of 2.5 N in hexanes, 100 mmol) to HMDS (22 mL, 104 mmol) in THF (23 mL) at -78 °C. [2-^{13}C]-Ethyl acetate (**30a**) (3.2g, 35.4 mmol) was added and the mixture was stirred at -65 °C for 30 min. [1-^{13}C]-Acetyl chloride (**31a**) (3.2 g, 40.3 mmol) was added over 30 min and the mixture was stirred at -65 °C for an additional 3.5 h. The reaction was quenched by addition of 2N HCl (290 mL) at -65 °C, and the solution was extracted with diethyl ether (4 × 150 mL). The combined ethereal layers were washed with 150 mL each of 1N HCl, saturated $NaHCO_3$, brine, and then dried (Na_2SO_4). After concentration *in vacuo* the resulting yellow residue was distilled to afford the product **32a** (4.4 g, 94%) as a mixture of keto:enol in a ratio of 96:4. The NMR data reported are for the keto form only. Bp 75-78 °C; IR (neat) 2985 (m), 1740 (s), 1680 (s), 1303 (s), 1139 (s) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.15 (q, 2H, J = 7.2 Hz, H-1'), 3.40 (dd, 2H, J = 130.2,

6.2 Hz, H-2), 2.20 (d, 3H, J = 6.2 Hz, H-4), 1.25 (t, 3H, J = 7.2 Hz, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.59 (d, J = 37.7 Hz, C-3), 172.44 (C-1), 61.30 (C-1'), 50.07 (d, J = 37.7 Hz, C-2), 26.89 (C-4), 14.02 (C-2'); MS (EI) calcd for $^{13}C_2C_4H_{10}O_3$ 132.0697, found 132.0700 (M^+, 94), 87.0361 (M-OEt, 100).



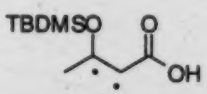
• = ^{13}C

Ethyl [2,3-$^{13}C_2$]-3-hydroxybutyrate (33a). To a solution of ethyl [2,3-$^{13}C_2$]-acetoacetate (**32a**) (5.0 g, 37.8 mmol) in ethanol (24 mL) at 0°C was added a solution of sodium borohydride (0.82 g, 21.8 mmol) in ethanol (20 mL) dropwise. The mixture was stirred at 0°C for 1.5 h and then at r.t. for 3.5 h. The reaction mixture was quenched by addition of 2N HCl (190 mL), and the solution was extracted with diethyl ether (6 × 250 mL). The combined ethereal layers were washed with brine and dried (Na_2SO_4). After evaporation *in vacuo*, the crude product was purified by silica gel column chromatography (50 to 100% diethyl ether in hexanes, R_f 0.20 with 50%) to give **33a** (5 g, 98%). IR (CH_2Cl_2 cast) 3445 (br s), 2977 (s), 1733 (s), 1287 (s), 1179 (s) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.20 (br d, 1H, J ~ 130 Hz, H-3), 4.15 (q, 2H, J = 7.1 Hz, H-1'), 2.80 (br s, 1H, OH), 2.45 (br d, 2H, J ~ 130 Hz, H-2), 1.25 (t, 3H, J = 7.1 Hz, H-2'), 1.22 (m, 3H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.13 (C-1), 64.25 (d, J = 37.8 Hz, C-3), 60.62 (C-1'), 42.86 (d, J = 37.8 Hz, C-2), 22.41 (C-4), 14.15 (C-2'); MS (EI) calcd for $^{13}C_2C_4H_{12}O_3$ 134.0854, found 134.0847 (M^+, 0.3), 119.0620 (M-Me, 45), 89.0536 (M-OEt, 100); Anal. Calcd for $C_6H_{12}O_3$: C, 54.53; H, 9.15. Found: C, 54.75; H, 9.39.



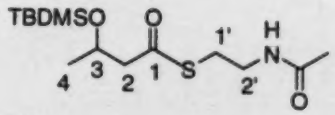
• = ^{13}C

Ethyl [2,3-$^{13}C_2$]-3-(*tert*-butyldimethylsilyloxy)butyrate (34a). A modification of the procedure of Liu *et al.*[5] was used. Ethyl [2,3-$^{13}C_2$]-3-hydroxybutyrate (**33a**) (5.0 g, 37.3 mmol), *tert*-butyldimethylsilyl chloride (8.2 g, 54.4 mmol), and imidazole (3.2 g, 47.0 mmol) were dissolved in DMF (25 mL). The mixture was stirred for 1.5 h before more DMF (9 mL) was added. The mixture was then stirred at r.t. for 1.5 days, at which time hexanes (275 mL) were added to dilute the solution which was then washed with water (3 × 275 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The crude oil was distilled under reduced pressure to afford **34a** as a colorless oil (5.0 g, 54%). Bp 90 °C (< 0.5 mm Hg); IR ($CHCl_3$ cast) 2930 (s), 1739 (s), 1179 (s), 1069 (s), 837 (s), 776 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 4.23 (br d, 1H, J ~ 130 Hz, H-3), 4.07 (dq, 2H, J = 7.2, 4.0 Hz, H-1'), 2.40 (br d, 1H, J ~ 130 Hz, H_a-2), 2.35 (br d, 1H, J ~ 130 Hz, H_b-2), 1.20 (t, 3H, J = 7.2 Hz, H-2'), 1.15 (m, 3H, H-4), 0.80 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.05 (s, $SiC\underline{H}_3$), 0.00 (s, $SiC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.17 (C-1), 65.51 (d, J = 43.1 Hz, C-3), 60.06 (C-1'), 44.87 (d, J = 43.1 Hz, C-2), 25.64 ($SiC(\underline{C}H_3)_3$), 23.82 (C-4), 14.11 (C-2'), -5.15 ($Si(\underline{C}H_3)_2$); MS (EI) calcd for $^{13}C_2C_{10}H_{26}O_3Si$ 247.1640, found 247.1642 (M-H, 0.5), 233.1484 (M-Me, 5), 191.1018 (M-C_4H_9, 100); Anal. Calcd for $C_{12}H_{26}SiO_3$: C, 58.49; H, 10.63. Found: C, 58.62; H, 10.63.



• = ^{13}C

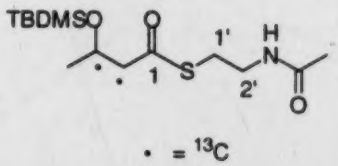
[2,3-$^{13}C_2$]-3-(*tert*-Butyldimethylsilyloxy)butyric acid (35a). A solution of NaOH (0.78 g, 19.5 mmol) in water (8.5 mL) was added over 20 min to a solution of ethyl [2,3-$^{13}C_2$]-3-(*tert*-butyldimethylsilyloxy)butyrate (**34a**) (2.2 g, 9.1 mmol) in a THF/water mixture (50 mL/12.4 mL respectively). The mixture was stirred at 45-50 °C for 3.5 h, then approximately half of the THF was evaporated *in vacuo*, and the mixture was heated further at 45-50 °C for 1.5 day. The THF was evaporated *in vacuo*, and the resulting aqueous solution was acidified to pH 1.5 (2N HCl) and extracted with chloroform (3 × 130 mL). The combined organic layers were dried (Na_2SO_4), concentrated *in vacuo* and used without further purification (1.4 g, 71%). IR ($CHCl_3$ cast) 3345 (br m), 2930 (s), 1712 (s), 1473 (m), 1408 (m), 1256 (s), 837 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.30 (br d, 1H, J ~ 130 Hz, H-3), 2.45 (br d, 2H, J ~ 130 Hz, H-2), 1.25 (m, 3H, H-4), 0.90 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.10 (s, 6H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.50 (C-1), 65.86 (d, J = 38.7 Hz, C-3), 44.97 (d, J = 38.7 Hz, C-2), 33.50 ($Si\underline{C}(CH_3)_3$), 25.63 ($SiC(\underline{C}H_3)_3$), 23.53 (C-4), -3.61 ($Si(\underline{C}H_3)_2$); MS (CI, NH_3) 238 (MNH_4^+, 20), 221 (MH^+, 100).



3-(*tert*-Butyldimethylsilyloxy)butyric acid, *N*-acetylcysteamine thioester (36). To a solution of 3-(*tert*-butyldimethylsilyloxy)butyric acid (**35**) (1.01 g, 4.6 mmol) in DMF (15 mL) at 0 °C were added diphenylphosphoryl azide (3.1 g, 11.2 mmol), triethylamine (4.0 mL, 28 mmol), and NAC (2.0 g, 17.2 mmol). The mixture was stirred at r.t. overnight. Diethyl ether (400 mL) was added to dilute the solution before washing with 300 mL each of 5% citric acid, saturated $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Purification by silica gel

chromatography (50% ethyl acetate in hexanes, R_f 0.38 with 100%) gave the thioester **36** as a yellow oil (1.37 g, 93%). IR ($CHCl_3$ cast) 3285 (br m), 2956 (s), 2930 (s), 1691 (s), 1655 (s), 1553 (m), 1256 (m), 1086 (s), 992 (s), 836 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.79 (br s, 1H, NH), 4.29 (m, 1H, H-3), 3.42 (dq, 2H, J = 5.6, 2.2 Hz, H-2'), 3.01 (m, 2H, H-1'), 2.59 (dd, 1H, J = 14.0, 7.0 Hz, H_a-2), 2.74 (dd, 1H, J = 14.0, 5.0 Hz, H_b-2), 1.95 (s, 3H, $C\underline{H}_3CON$), 1.19 (d, 3H, J = 6.2 Hz, H-4), 0.86 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.06 (s, 3H, $SiC\underline{H}_3$), 0.03 (s, 3H, $SiC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.98 (C-1), 170.186 ($\underline{C}ON$), 65.97 (C-3), 53.84 (C-2), 39.59 ($\underline{C}H_3CON$), 28.64 (C-1'), 25.72 ($SiC(CH_3)_3$), 23.82 (C-2'), 23.18 (C-4), 14.18 ($SiC(\underline{C}H_3)_3$), -4.52 ($Si\underline{C}H_3$), -5.05 ($Si\underline{C}H_3$); MS (CI, NH_3) 337 (MNH_4^+, 30), 320 (MH^+, 100); Anal. Calcd for $C_{14}H_{29}NO_3SSi$: C, 52.62; H, 9.15; N, 4.38. Found: C, 52.41; H, 9.04; N, 4.03.

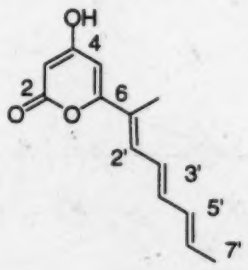
Alternative procedure for the preparation of 3-(*tert*-butyldimethylsilyloxy)butyric acid, *N*-acetylcysteamine thioester (36). A modification of the procedure of Liu *et al.*[5] was used. A solution of 3-(*tert*-butyldimethylsilyloxy)butyric acid (**35**) (0.44 g, 2.0 mmol) in dichloromethane (15 mL) containing molecular sieves was cooled to –18 °C. Simultaneously, a solution of NAC (0.28 g, 2.3 mmol) in dichloromethane (5 mL), and a solution of DCC (0.53 g, 2.5 mmol) and DMAP (43 mg, 0.3 mmol) in dichloromethane (5 mL) were added. The reaction was allowed to warm to r.t. then stirred overnight. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel flash chromatography (40% hexanes in ethyl acetate, R_f 0.24). The product **36** was obtained as a colorless oil (0.56 g, 88%) with identical spectral data as above.



[2,3-$^{13}C_2$]-3-(*tert*-Butyldimethylsilyloxy)butyric acid, *N*-acetylcysteamine thioester (36a). To a solution of [2,3-$^{13}C_2$]-3-(*tert*-butyldimethylsilyloxy)butyric acid (**35a**) (0.72 g, 3.28 mmol) in DMF (12 mL) at 0 °C were added diphenylphosphoryl azide (2.4 g, 8.7 mmol), triethylamine (3.1 mL, 22 mmol), and NAC (1.4 mL, 13.4 mmol). The mixture was stirred at r.t overnight. Diethyl ether (315 mL) was added to dilute the solution before washing with 235 mL each of 5% citric acid, saturated $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. Purification by silica gel column chromatography (50% ethyl acetate in hexanes, R_f 0.38 with 100%) gave the thioester **36a** as a yellow oil (0.25 g, 25%). IR ($CHCl_3$ cast) 3291 (br m), 2929 (s), 1690 (s), 1657 (s), 1554 (s), 1110 (s), 1002 (s), 987 (s), 837 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.90 (br s, 1H, NH), 4.30 (br d, 1H, J ~ 130 Hz, H-3), 3.40 (dt, 2H, J = 6.5, 2.2 Hz, H-1'), 3.00 (q, 2H, J = 6.5 Hz, H-2'), 2.75 (br d, 1H, J ~ 130 Hz, H_a-2), 2.65 (br d, 1H, J ~ 130 Hz, H_b-2), 1.95 (s, 3H, NCOC$\underline{H}_3$) 1.15 (m, 3H, H-4), 0.85 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.05 (s, 3H, SiC$\underline{H}_3$), 0.02 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.04 (C$\underline{O}$S), 170.22 (C$\underline{O}$N), 66.02 (d, J = 37.8 Hz, C-3), 53.89 (d, J = 37.8 Hz, C-2), 39.66 (C-1'), 25.72 (Si$\underline{C}$(CH$_3$)$_3$), 28.71 (C-2'), 25.78 (SiC(C$\underline{H}_3$)$_3$), 23.87 (C-4), 23.24 (C$\underline{H}_3$CO), -4.46 (C$\underline{H}_3$Si), -4.99 (C$\underline{H}_3$Si); MS (CI, NH_3) 339 (MNH_4^+, 63), 322 (MH^+, 100); MS (EI) calcd for $^{13}C_2C_{12}H_{29}NO_3SSi$ 264.1000, found 264.1000 (M-C_4H_9, 25), 86.0604 ($C_4H_8NO^+$, 100).

[1,2-$^{13}C_2$]-Acetyl CoA (39a). Methods described elsewhere[173] were modified for the synthesis of [1,2-$^{13}C_2$]-acetyl CoA (**39a**). To a suspension of sodium [1,2-$^{13}C_2$]-acetate (0.25 g, 2.9 mmol) in THF (15 mL) cooled to 2 °C was added ethyl chloroformate (0.3 mL, 3.1 mmol). The reaction mixture was stirred at r.t. for two hours, then heated under reflux overnight. The reaction was followed by IR ($\nu_{acetate\ CO}$ = 1604 cm^{-1}, $\nu_{chloroformate\ CO}$ = 1780 cm^{-1}, $\nu_{anhydride\ CO}$ = 1818, 1750 cm^{-1}). The mixture was filtered through a sintered-glass funnel to remove the unreacted acetate. The filtrate

was slowly added to a solution of coenzyme A (50 mg, 0.065 mmol) in water (10 mL) at pH adjusted to 7.5 with sodium hydrogen carbonate and cooled to 0 °C. The reaction was followed using the sodium nitroprusside test. The reaction was completed in less than ten minutes. The THF was evaporated *in vacuo* and the resulting aqueous solution was freeze-dried for one day. Extraction in water and freeze-drying afforded product **39a** (137 mg crude). IR (microscope) 3327 (br s), 1651 (s), 1238 (s), 1119 (s), 1074 (s), 944 (s) cm^{-1}; MS (ES) calcd for $^{13}C_2C_{21}H_{39}N_7O_{17}P_3S$ 812.1403, found 812.1418 (MH_5^+, 85), 834.1 (MH_4Na^+, 44), 856.1 ($MH_3Na_2^+$, 45), 878.0 ($MH_2Na_3^+$, 45), 900.0 ($MHNa_4^+$, 35). MS (ES) found for $C_{23}H_{39}N_7O_{17}P_3S$ 810.1 (MH_5^+, 100), 832.1 (MH_4Na^+, 70), 854.1 ($MH_3Na_2^+$, 35), 876.1 ($MH_2Na_3^+$, 45), 900.0 ($MHNa_4^+$, 35).



4-Hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (41). Compound **41** was isolated or detected as described in the fermentation section for the *A. nidulans lovB*, *A. nidulans lovB+lovC*, and *A. terreus lovC* strains. The yield was approximately 10 mg of unstable yellow powder from 6 L of *A. nidulans lovB*. Mp/decomposition: 185-195 °C; UV (MeOH solution) λmax 356, 268, 218 nm; IR (CH_2Cl_2 cast) 3100 (br s), 1683 (br s), 1662 (s), 1621 (s), 1549 (s) cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 7.08 (d, 1H, *J* = 10.4 Hz, H-2'), 6.50-6.58 (m, 2H, H-3', H-4'), 6.26 (tdd, 1H, *J* = 15.0, 9.2, 2.0 Hz, H-5'), 6.17 (d, 1H, *J* = 1.6 Hz, H-5), 5.92 (m, 1H, H-6'), 5.38 (d, 1H, *J* = 1.6 Hz, H-3), 1.99 (s, 3H, 1'-Me), 1.81 (d, 3H, *J* = 5.7 Hz, H-7'); ^{13}C

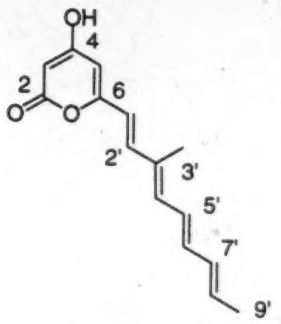
NMR (125 MHz, CD_3OD) δ 173.0 (C-4), 167.0 (C-2), 162.0 (C-6), 140.53 (C-4'), 134.32 (C-6'), 133.76 (C-2'), 133.26 (C-5'), 126.74 (C-3'), 99.63 (C-5), 90.0 (br, C-3, C-1'), 18.52 (C-7'), 12.52 (1'-Me); MS (ES) calcd for $C_{13}H_{15}O_3$ 219.1021, found 219.1023 (MH^+, 100).

^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (41a) from incorporation of sodium [1,2-$^{13}C_2$]-acetate. Compound **41a** was labeled by feeding sodium [1,2-$^{13}C_2$]-acetate to cultures of the *A. nidulans lovB* strain and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **41a** were the same as for **41** except for the following. ^{1}H NMR for labeled compound in 70% labeled mixture (600 MHz, CD_2Cl_2) δ 7.08 (br d, 1H, J_{CH} ~ 150 Hz, H-2'), 6.52 (br d, 2H, J_{CH} ~ 154 Hz, H-3' and H-4'), 6.23 (br d, 1H, J_{CH} ~ 154 Hz, H-5'), 6.15 (br d, 1H, J_{CH} ~ 170 Hz, H-5), 5.90 (br d, 1H, J_{CH} ~ 154 Hz, H-6'), 5.50 (br d, 1H, J_{CH} ~ 145 Hz, H-3), 1.95 (s, 3H, 1'-Me), 1.81 (br d, 3H, J_{CH} ~ 126Hz, H-7'); ^{13}C NMR for ^{13}C-labeled sample (125 MHz, CD_3OD) δ 173.5 (dt, J_{CC} ~ 65, 29 Hz, C-4), 167.5 (d, J_{CC} ~ 81 Hz, C-2), 163.5 (m, C-6), 140.5 (br t, J_{CC} ~ 63 Hz, C-4'), 134.0 (br t, J_{CC} ~ 45 Hz, C-6'), 133.0 (m, C-2'), 132.0 (m, C-5'), 126.0 (br t, J_{CC} = 63 Hz, C-3'), 99.5 (m, C-5), 90.5 (m, C-3, C-1'), 18.5 (d, J_{CC} = 43 Hz, C-7'), 12.0 (weak d, J_{CC} = 50 Hz, 1'-$\underline{C}H_3$); MS (ES) 461 ($2MNa^+$, 45), 231 (MH^+, 100).

^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (41b) from incorporation of sodium [1-^{13}C]-acetate. Compound **41b** was labeled by feeding sodium [1-^{13}C]-acetate to cultures of the *A. nidulans lovB* strain and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **41b** were the same as for **41** except for the following. ^{1}H NMR for 70% labeled mixture (600MHz, CD_2Cl_2) δ

7.08 (br d, 0.7H, J_{CH} ~ 150 Hz, H-2' on ^{13}C), 7.10 (m, 0.3H, H-2'), 6.50 (br d, 0.7H, J_{CH} ~ 154 Hz, H-4' on ^{13}C), 6.42-6.58 (m, 1.3H, H-3' and H-4'), 6.23 (m, 1H, H-5'), 6.13 (d, 1H, J = 1.6 Hz, H-5), 5.92 (br d, 0.7H, J_{CH} ~ 154 Hz, H-6' on ^{13}C), 5.88-5.95 (m, 0.3H, H-6'), 5.55 (d, 1H, J = 1.6 Hz, H-3), 1.95 (s, 3H, 1'-Me), 1.82 (d, 3H, J = 5.7 Hz, H-7'); ^{13}C NMR (125 MHz, CD_3OD) δ 173.0 (C-4), 167.0 (C-2), 162.0 (C-6), 140.53 (C-4'), 134.32 (C-6'), 133.76 (C-2'); MS (ES) 225 (MH^+, 100).

^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (41c) from incorporation of sodium [2-^{13}C]-acetate. Compound **41c** was labeled by feeding sodium [2-^{13}C]-acetate to cultures of the *A. nidulans lovB* strain and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **41c** were the same as for **41** except for the following. ^1H NMR for 70% labeled compound (600 MHz, CD_2Cl_2) δ 7.07 (d, 1H, J = 10.4 Hz, H-2'), 6.50 (br d, 0.7H, J_{CH} ~ 154 Hz, H-3' on ^{13}C), 6.42-6.58 (m, 1.3H, H-3', H-4'), 6.23 (br d, 0.7H, J_{CH} ~ 154 Hz, H-5' on ^{13}C), 6.23 (m, 0.3H, H-5'), 6.15 (br d, 0.7H, J_{CH} ~ 170 Hz, H-5 on ^{13}C), 6.13 (d, 0.3H, J = 1.6 Hz, H-5), 5.88-5.95 (m, 1H, H-6'), 5.55 (br d, 0.7H, J_{CH} ~ 145 Hz, H-3 on ^{13}C), 5.55 (d, 0.3H, J = 1.6 Hz, H-3), 1.95 (s, 3H, 1'-Me), 1.82 (br d, 2.1H, J_{CH} ~ 126Hz, H-7' on ^{13}C), 1.82 (d, 0.9H, J = 5.7 Hz, H-7'); ^{13}C NMR (125 MHz, CD_3OD) δ 133.26 (C-5'), 126.74 (C-3'), 99.63 (C-5), 90 (C-3, C-1'), 18.52 (C-7'); MS (ES) 225 (MH^+, 100).



4-Hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (42). Compound **42** was isolated or detected as described in the fermentation section from the *A. nidulans lovB*, *A. nidulans lovB+lovC*, and *A. terreus lovC* strains. The yield was approximately 10 mg of unstable yellow powder from 6 L of *A. nidulans lovB*. Mp/decomposition: 165-170 °C; UV (MeOH solution) λmax 378, 288, 282, 232 nm; IR (CH_2Cl_2 cast) 3050 (br s), 1675 (br s), 1651 (s), 1625 (s), 1541 (s) cm^{-1}; ^1H NMR (600 MHz, CD_2Cl_2) δ 7.12 (d, 1H, *J* = 15.5 Hz, H-2'), 6.49 (dd, 1H, *J* = 15.0, 2.0 Hz, H-5'), 6.40 (m, 1H, H-6'), 6.32 (m, 1H, H-4'), 6.21 (ddd, 1H, *J* =15.0, 12.5, 2.0 Hz, H-7'), 6.19 (d, 1H, *J* = 15.5 Hz, H-1'), 6.05 (d, 1H, *J* = 1.8 Hz, H-5), 5.82 (m, 1H, H-8'), 5.33 (d, 1H, *J* = 1.8 Hz, H-3), 1.90 (s, 3H, 3'-Me), 1.77 (d, 3H, *J* = 7.1 Hz, H-9'); ^{13}C NMR (125 MHz, CD_3OD) δ 173.18 (C-4), 167.58 (C-2), 162.03 (C-6), 141.40 (C-2), 138.80 (C-6'), 138.28 (C-4'), 134.60 (C-2'), 133.49 (C-7'), 133.05 (C-8'), 127.60 (C-5'), 118.39 (C-1'), 102.07 (C-5), 90.0 (C-3), 18.50 (C-9'), 12.37 (3'-Me); MS (ES) calcd for $C_{15}H_{17}O_3$ 245.1178, found 245.1130 (MH^+, 100).

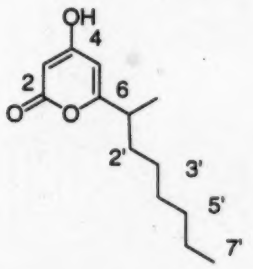
^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (42a) from incorporation of sodium [1,2-$^{13}C_2$]-acetate. Compound **42a** was labeled by feeding sodium [1,2-$^{13}C_2$]-acetate to cultures of the *A. nidulans lovB* strain and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **42a**

were the same as for **42** except for the following. ^{1}H NMR for labeled compound in 70% labeled mixture (600 MHz, CD_2Cl_2) δ 7.15 (br d, 1H, J_{CH} ~ 151 Hz, H-2'), 6.47 (br d, 1H, J ~ 125 Hz, H-5'), 6.5-6.0 (m, 4H, H-6', H-4', H-7', H-1'), 6.01 (br d, 1H, J_{CH} ~ 165 Hz, H-5), 5.86 (br d, 1H, J_{CH} ~ 151 Hz, H-8'), 5.52 (br d, J_{CH} ~ 168 Hz, H-3), 1.92 (s, 3H, 3'-Me), 1.81 (br d, 3H, J_{CH} ~ 175 Hz, H-9'); ^{13}C NMR for ^{13}C-labeled sample (125 MHz, CD_3OD) δ 173.0 (br t, J_{CC} ~ 65 Hz, C-4), 167.5 (d, J_{CC} = 80 Hz, C-2), 161.5 (m, C-6), 141.0 (m, C-2'), 138.8 (m, C-6'), 138.3 (m, C-4'), 135.0 (m, C-3'), 133.5 (m, C-7'), 132.0 (m, C-8'), 127.5 (m, C-5'), 119.0 (m, C-1'), 102.0 (m, C-5), 90.0 (m, C-3), 18.5 (d, J_{CC} ~ 45 Hz, C-9'), 12.0 (weak d, J_{CC} ~ 45 Hz, 3'-Me); MS (ES) 515 ($2MH^+$, 45), 259 (MH^+, 100).

^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (42b) from incorporation of sodium [1-^{13}C]-acetate. Compound **42b** was labeled by feeding sodium [1-^{13}C]-acetate to cultures of the *A. nidulans lovB* strain and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **42b** were the same as for **42** except for the following. ^{1}H NMR for 70% labeled compound (600 MHz, CD_2Cl_2) δ 7.15 (br d, 0.7H, J_{CH} ~ 151 Hz, H-2' on ^{13}C), 7.15 (m, 0.3H, H-2'), 6.47 (m, 1H, H-5'), 6.5-6.0 (m, 4H, H-6', H-4', H-7', H-1'), 6.01 (d, 1H, J = 1.8 Hz, H-5), 5.86 (br d, 0.7H, J_{CH} ~ 151 Hz, H-8' on ^{13}C), 5.86 (m, 0.3H, H-8'), 5.52 (d, 1H, J = 1.8 Hz, H-3), 1.92 (s, 3H, 3'-Me), 1.81 (d, 3H, J = 7.1 Hz, H-9'); ^{13}C NMR (125 MHz, CD_3OD) δ 173.18 (C-4), 167.58 (C-2), 162.03 (C-6), 141.40 (C-2), 138.80 (C-6'), 138.28 (C-4'), 133.05 (C-8'); MS (ES) 252 (MH^+, 100).

^{13}C-Labeled 4-Hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (42c) from incorporation of sodium [2-^{13}C]-acetate. Compound **42c** was labeled by feeding sodium [2-^{13}C]-acetate to cultures of the *A. nidulans lovB* strain

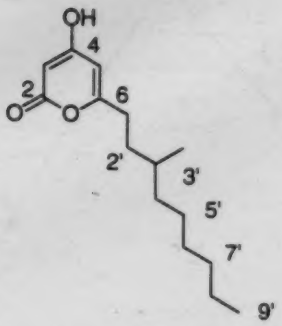
and isolated as described in the fermentation section. The yield was approximately 10 mg of unstable yellow powder from 6 L of culture. The data for **42c** were the same as for **42** except for the following. ^{1}H NMR for the 70% labeled compound (600 MHz, CD_2Cl_2) δ 7.15 (d, 1H, J = 15.5 Hz, H-2'), 6.47 (br d, 0.7H, J_{CH} ~ 125 Hz, H-5' on ^{13}C), 6.47 (m, 0.3H, H-5'), 6.5-6.0 (m, 4H, H-6', H-4', H-7', H-1'), 6.01 (br d, 0.7H, J_{CH} ~ 165 Hz, H-5 on ^{13}C), 6.01 (d, 0.3H, J = 1.8 Hz, H-5), 5.86 (m, 1H, H-8'), 5.52 (br d, 0.7H, J_{CH} ~ 168 Hz, H-3 on ^{13}C), 5.52 (d, 0.3H, J = 1.8 Hz, H-3), 1.92 (s, 3 H, 3'-Me), 1.81 (br d, 2.1H, J_{CH} ~ 175 Hz, H-9' on ^{13}C), 1.81 (d, 0.9H, J = 7.1 Hz, H-9'); ^{13}C NMR (125 MHz, CD_3OD) δ 134.60 (C-3'), 133.49 (C-7'), 127.60 (C-5'), 118.39 (C-1'), 102.07 (C-5), 90.0 (C-3), 18.50 (C-9'); MS (ES) 252 (MH^+, 100).



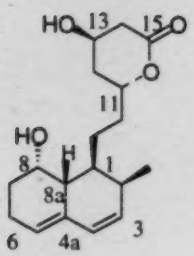
4-Hydroxy-6-(1-methylhept-1-yl)-2-pyrone (43). To a suspension of Pd/C (ca. 1 mg) in methanol (2 mL) under hydrogen atmosphere was added a solution of unlabeled **41** (10 mg, 0.045 mmol) in methanol (5 mL). The mixture was stirred at r.t. under hydrogen for ca. 14 hours. The catalyst was removed by filtration through sintered glass. The filtrate was evaporated, and purification was accomplished by HPLC using the same conditions as for compounds **41** and **42** except that the UV detector was set at 220 nm. The major component (retention time of 16 min) was collected to afford 2 mg (20%) of **43** as an oil. ^{1}H NMR (600 MHz, CD_3OD) δ 5.96 (d, 1H, J = 2.1 Hz, H-5), 5.33 (d, 1H, J = 2.1 Hz, H-3), 2.59 (m, 1H, H-1'), 1.63 (m, 1H,

H_a-2'), 1.50 (m, 1H, H_b-2'), 1.34-1.14 (m, 8H, H-3', H-4', H-5', H-6'), 1.20 (m, 3H, 1'-Me), 0.89 (t, 3H, J = 7.0 Hz, H-7'); ^{13}C NMR (125 MHz, CD_3OD) δ 173.4 (C-4), 172.1 (C-6), 168.5 (C-2), 100.8 (C-5), 89.5 (C-3), 39.6 (C-1'), 35.5 (C-2'), 32.8 (C-5'), 30.2 (C-4'), 28.3 (C-3'), 23.6 (C-6'), 18.7 (1'-$\underline{C}H_3$), 14.3 (C-7'); MS (ES) calcd for $C_{13}H_{21}O_3$ 225.1491, found 225.1486 (MH^+, 100).

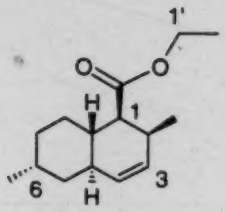
^{13}C-Labeled 4-Hydroxy-6-(1-methylhept-1-yl)-2-pyrone (43a) from incorporation of sodium [1,2-$^{13}C_2$]-acetate. Compound **43a** was prepared using the same procedure as for **43**. Thus, **41a** (10 mg, 0.045 mmol) was treated with Pd/C (ca. 1 mg) in methanol (7 mL) under a hydrogen atmosphere to afford 2 mg (20%) of **43a**. ^{1}H NMR of labeled compound in 70% labeled mixture (600 MHz, CD_3OD) δ 5.96 (dd, 1H, J = 2.1 Hz, J_{CH} = 150 Hz, H-5), 5.33 (dd, 1H, J = 2.1 Hz, J_{CH} = 163 Hz, H-3), 2.59 (br d, 1H, J_{CH} = 130 Hz, H-1'), 1.63 (br d, 1H, J_{CH} = 120 Hz, H_a-2'), 1.50 (m, 1H, J_{CH} = 120 Hz, H_b-2'), 1.34-1.14 (m, 11H, H-3', H-4', H-5', H-6', 1'-Me), 0.89 (td, 3H, J = 7.0 Hz, J_{CH} = 120 Hz, H-7'); ^{13}C NMR for 70% labeled compound (125 MHz, CD_3OD) δ 173.4 (br dd, J_{CC} ~ 68, 58 Hz, C-4), 172.1 (br dd, J_{CC} ~ 74, 50 Hz, C-6), 168.5 (br d, J_{CC} ~ 79 Hz, C-2), 100.8 (br dd, J_{CC} ~ 74, 58 Hz, C-5), 89.5 (m, C-3), 39.6 (m, C-1'), 35.5 (br t, J_{CC} ~ 35 Hz, C-2'), 32.8 (br t, J_{CC} ~ 34 Hz, C-5'), 30.2 (br t, J_{CC} ~ 35 Hz, C-4'), 28.3 (br t, J_{CC} ~ 35 Hz, C-3'), 23.6 br dt, J_{CC} ~ 35, 4 Hz, C-6'), 18.7 (br d, J_{CC} ~ 34 Hz, 1'-$\underline{C}H_3$), 14.3 (br dd, J_{CC} ~ 34, 4 Hz, C-7'); MS (ES) 237 (MH^+, 100).



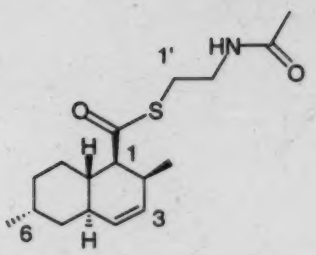
^{13}C-Labeled 4-Hydroxy-6-(3-methyl-non-1-yl)-2-pyrone (44a) from incorporation of sodium [1,2-$^{13}C_2$]-acetate. To a suspension of Pd/C (ca. 1 mg) in methanol (2 mL) under a hydrogen atmosphere was added a solution of ^{13}C-labeled **42a** (10 mg, 0.040 mmol) in methanol (5 mL). The mixture was stirred at 20 °C under hydrogen for ca. 14 hours. The catalyst was removed by filtration through sintered glass. The filtrate was evaporated, and purification was accomplished by HPLC using the same conditions as for compounds **41** and **42**, except that UV detector was set at 280 nm. The major component (retention time of 23 min) was collected to afford 3 mg (30%) of **44a** as an oil. ^{1}H NMR for labeled compound in 70% labeled mixture (600 MHz, CD_3OD) δ 5.97 (d, 1H, J_{CH} = 171 Hz, H-5), 5.32 (d, 1H, J_{CH} = 171 Hz, H-3), 2.5 (m, 1H, H-3'), 1.55 (m, 2H, H-6'), 1.42 (m, 4H, H-4', H-5'), 1.27 (m, 4H, H-1', H-7'), 1.30 (m, 4H, H-2', H-8'), 0.92 (m, 6H, H-9', 3'-C$\underline{H}_3$); ^{13}C NMR for 70% labeled compound (125 MHz, CD_3OD) δ 171.5 (m, C-4), 168.5 (m, C-2), 164.0 (m, C-6), 101.1 (br t, J_{CC} ~ 67 Hz, C-5), 89.6 (m, C-3), 37.5 (t, J_{CC} = 34.8 Hz, C-1'), 34.7 (t, J_{CC} = 34.4 Hz, C-6'), 33.4, 32.8 and 32.2 (C-3', C-4', C-5'), 30.4 (t, J_{CC} = 34.4 Hz, C-7'), 27.7 (t, J_{CC} = 34.8 Hz, C-2'), 23.1 (t, J_{CC} = 34.4 Hz, C-8'), 19.2 (d, J_{CC} = 35.2 Hz, 3'-$\underline{C}H_3$), 14.0 (d, J_{CC} = 34.4 Hz, C-9'); MS (ES) calcd for $C_{15}H_{27}O_3$ 255.1960, found 255.1959 (MH^+, 100).



6-Desmethylmonacolin J (52). A solution of compactin (**5**) (55 mg, 0.13 mmol) and lithium hydroxide monohydrate (60 mg, 1.3 mmol) in water (15 mL) was heated under reflux for one day. The reaction mixture was cooled in an ice bath, acidified to pH 1 (2N HCl), and extracted with both ethyl acetate (5 × 30 mL) and chloroform (4 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. In order to ensure that all the 6-desmethylmonacolin J (**52**) was lactonized, toluene (60 mL) was added to the residue and the solution was heated under reflux with a sohxlet containing calcium hydride for 1 hour. The toluene was removed *in vacuo* and the compound was purified by silica gel flash column chromatography (100% ethyl acetate, R_f 0.12), affording the product **52** as a colorless oil (30 mg, 75%). $[\alpha]_D^{25}$ = +78 (*c* 0.15, $CHCl_3$); IR ($CHCl_3$ cast) 3407 (br s), 2928 (s), 1710 (s), 1256 (s), 1075 (s), 754 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (d, 1H, *J* = 9.6 Hz, H-4), 5.71 (dd, 1H, *J* = 9.6, 6.0 Hz, H-3), 5.52 (br d, 1H, *J* = 2.1 Hz, H-5), 4.69 (m, 1H, H-11), 4.52 (m, 1H, H-13), 4.21 (br s, 1H, H-8), 2.67 (dd, 1H, *J* = 17.7, 5.0 Hz, H_a-14), 2.59 (ddd, 1H, *J* = 17.7, 3.7, 1.7 Hz, H_b-14), 2.31 (m, 2H, H-2, H_a-6), 2.14 (m, 2H, H_b-6, H-8a), 1.96 (m, 2H, H_a-7, H_a-12), 1.82-1.62 (m, 5H, H-1, H_b-7, H_a-9, H_a-10, H_b-12), 1.53-1.39 (m, 2H, H_b-9, H_b-10), 0.88 (d, 3H, *J* = 7.0 Hz, 2-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.81 (C-15), 133.27 (C-4a), 133.01 (C-3), 128.33 (C-4), 123.65 (C-5), 76.22 (C-11), 64.42 (C-8), 62.56 (C-13), 38.75 (C-8a), 38.51 (C-14), 36.39 (C-1), 36.16 (C-7), 32.63 (C-9), 30.77 (C-2), 29.09 (C-12), 23.79 (C-10), 20.29 (C-6), 13.87 (2-Me); MS (ES) calcd for $C_{18}H_{26}O_4Na$ 329.1729, found 329.1725 (MNa^+, 100), 307.2 (MH^+, 10).

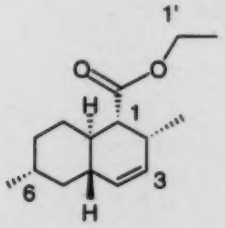


Ethyl (1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethylnaphthalene-1-carboxylate (55). In a separate experiment, the residue containing crude **56** obtained after work up of the LNKS-catalyzed Diels-Alder reaction described below was azeotropically dried by distillation of toluene (2 × 10 mL). A solution of the crude NAC ester **56** (~ 8 mg, 0.026 mmol) in dichloromethane (0.86 mL) was added to a solution of sodium ethoxide (3.5 mg, 0.052 mmol) in ethanol (0.59 mL). The mixture was stirred at r.t for one hour and then at 60 °C for 7 hours. The reaction mixture was diluted with water (ca. 2 mL) at 0 °C and extracted with chloroform (6 × 10 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. This crude extract was compared by TLC and HPLC to the three standards prepared by Dr. David J. Witter[46] for the two Diels-Alder adducts produced by spontaneous cyclization of the ethyl ester analogue of **16**, and the synthetic compound **55**. The reaction yielded these three Diels-Alder adducts in the following order of abundance: *endo* chemical adduct, *endo* enzyme adduct **55**, and *exo* chemical adduct. Purification by silica gel column chromatography (2% diethyl ether in hexanes) afforded the ester **55** (0.1 mg, > 5% and R_f 0.25 for **55**, R_f 0.24 and 0.23 for the two chemical adducts). The ester obtained had the same properties (TLC, HPLC, IR and MS) as the authentic material **55**.[46] IR (CD_2Cl_2 cast) 2924 (s), 1738 (s), 1260 (m) cm^{-1}; MS (EI) calcd for $C_{15}H_{24}O_2$ 236.1776, found 236.1788 (M^+, 21), 163.1486 (M-CO_2Et, 62), 162.1407 (M-HCO_2Et, 55), 81.0704 ($C_6H_9^+$, 100).



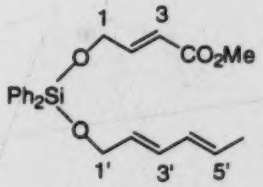
(1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2-4a,5,6,7,8a-Octahydro-2,6-dimethylnaphthalene-1-carboxylic acid, *N*-acetylcysteamine thioester (56). A solution of triene **16** (5 mg, 0.016 mmol) in methanol (100 µL) was added to a solution of the LNKS enzyme purified as in the cell free extract/enzyme purification section (60 µL of ca. 3 mg/mL solution in pellet buffer) diluted with more pellet buffer (2 mL). The resulting milky solution was allowed to react on an orbital shaker at r.t. and 50 rpm overnight. After dilution in water (ca. 3 mL) and neutralization, the mixture was extracted in chloroform (5 × 6 mL). Combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. The residue containing crude **56** was used directly in the transesterification described below, or purified by HPLC. The HPLC trace showed production of the two Diels-Alder adducts resulting from spontaneous cyclization (standards prepared by Dr. David J. Witter[46] and injected under the same conditions), as well as **56**. The column used was a µBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 µm, 125 Å (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was raised from 30% acetonitrile in water and 0.1% TFA, to 40% over 18 min, then raised from 40% to 49% over 4 min, raised from 49% to 100% over 3 min, and kept at 100% for 5 min. The UV detector was set at 200 nm. The two chemical adducts had retention times of 22.9 and 24.5 min. Compound **56** (<0.1 mg) was obtained as a white solid of retention time 19.9 min. $[\alpha]_D^{25} = -18$ (*c* 0.05, $CHCl_3$); IR ($CHCl_3$ cast) 3286 (br m), 2925 (s), 1685 (s), 1654 (s), 1094 (s), 1019 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.98 (m, 1H, H-3), 5.75 (br s, 1H, NH), 5.52 (m, 1H, H-4), 3.63 (br dd, 1H, *J* = 10.8, 4.9

Hz, H-8a), 3.42 (ddd, 1H, J = 8.8, 6.2, 2.7 Hz, H-2'), 3.04 (br t, 1H, J = 8.8 Hz, H-1'), 2.80 (m, 1H, H-1), 2.65 (m, 1H, H-2), 1.95 (s, 3H, NHCOC$\underline{H}_3$), 1.71 (br d, 1H, J = 6.6 Hz, H-4a), 1.61-1.44, 1.28-1.21 (2 m, 7H, H_a-5, H-6, H-7, H-8), 0.87 (m, 1H, H_b-5), 0.85 (d, 3H, J = 6.6 Hz, 2-Me), 0.83 (d, 3H, J = 6.8 Hz, 6-Me); MS (EI) calcd for $C_{17}H_{27}NO_2S$ 309.1762, found 309.1769 (M^+, 5), 190.1360 (M-NAC, 51), 163.1486 ($C_{12}H_{19}^+$, 56), 120.0482 ($NACH^+$, 58), 81.0704 ($C_6H_9^+$, 100).



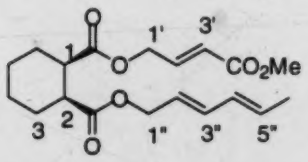
Ethyl (1*R*,2*R*,4a*S*,6*R*,8a*R*)-1,2,4a,5,6,7,8,8a-octahydro-2,6-dimethylnaphthalene-1-carboxylate (58). To a solution of the thioester **57** (5 mg, 0.016 mmol) in dichloromethane (0.52 mL) was added a solution of sodium ethoxide (1.65 mg, 0.024 mmol) in ethanol (0.28 mL) dropwise. The reaction mixture was heated at 60 °C for one hour and 50 °C overnight. The reaction was quenched by adding water (10 mL) at 0 °C, and extracted with chloroform (6 × 5 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. Purification by silica gel column chromatography (3% diethyl ether in hexanes, R_f 0.25) gave **58** (2.1 mg, 55%). Compound **58** had the same spectroscopic data as the standard described before.[46] $[\alpha]_D^{25}$ = -80 (*c* 0.8, $CHCl_3$), lit. $[\alpha]_D^{25}$ = -89.2 (*c* 1.40, $CHCl_3$);[46] IR (CH_2Cl_2 cast) 2953 (s), 2916 (s), 2871 (s), 2849 (s), 1735 (s), 1140 (s), 1082 (s) cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 5.53 (ddd, 1H, J = 9.7, 4.2, 2.4 Hz, H-3), 5.38 (m, 1H, H-4), 4.13 (q, 2H, J = 7.1 Hz, H-1'), 2.55 (m, 1H, H-1), 2.53 (m, 1H, H-2), 1.95 (m, 1H, H-8eq), 1.77-1.68 (m, 3H, H-4a, H-7eq, H-5eq), 1.52-1.43 (m, 1H, H-6), 1.35 (m, 1H, H-8a), 1.27 (t, 3H, J =

7.2 Hz, H-2'), 1.04 (dddd, 1H, J = 12.0, 12.0, 12.0, 3.0 Hz, H-8ax), 1.00-0.95 (m, 1H, H-7ax), 0.93 (d, 3H, J = 6.5 Hz, 2-Me), 0.90 (d, 3H, J = 6.9 Hz, 6-Me), 0.80 (m, 1H, H-5ax); MS (EI) calcd for $C_{15}H_{24}O_2$ 236.1776, found 236.1778 (M^+, 18), 191.14315 (M-OEt, 9), 190.13589 (M-EtOH, 9), 163.14834 (M-CO_2Et, 96), 163.14108 (M-HCO_2Et, 100).



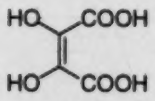
[3-Carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (59). The method of Ainsworth *et al.*[52a] was used. To a mixture of dichlorodiphenylsilane (3.1 mL, 31 mmol) and triethylamine (4.3 mL, 31 mmol) was added a solution of methyl (*E*)-4-hydroxy-2-butenoate (**64**) (1.98 g, 17 mmol) and (*E*,*E*)-2,4-hexadien-1-ol (1.7 mL, 14.5 mmol) in dichloromethane (12.6 mL). The reaction was exothermic and a precipitate formed. More dichloromethane (4.4 mL) was added and the mixture was stirred for one hour. Petroleum ether (200 mL) was added and the white solid was filtered and the solvent was evaporated *in vacuo*. The product was purified by Florisil column chromatography (1-10% diethyl ether in petroleum ether, R_f 0.52 at 30%) to give compound **59** as a colorless oil (1.9 g, 32%). IR ($CHCl_3$ cast) 3024 (w), 2852 (w), 1724 (s), 1430 (m), 1126 (s), 720 (s), 700 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (m, 4H, *ortho*-Ar), 7.45 (m, 6H, meta and para-Ar), 7.00 (td, 1H, J = 15.5, 3.6 Hz, H-2), 6.25 (td, 1H, J = 15.5, 2.2 Hz, H-3), 6.20 (m, 1H, H-3' or H-4'), 6.05 (m, 1H, H-3' or H-4'), 5.65 (m, 2H, H-2', H-5'), 4.50 (dd, 2H, J = 3.6, 2.2 Hz, H-1), 4.35 (d, 2H, J = 5.6 Hz, H-1'), 3.75 (s, 3H, $CO_2C\underline{H}_3$), 1.75 (d, 3H, J = 6.6 Hz, H-6'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.92 ($\underline{C}O_2CH_3$), 146.51 (C-2) 134.86 (*ortho*-Ar),

131.99 (C-2'), 131.46 (C-3'), 130.80, (ArC-Si), 130.56 (para-Ar), 129.75 (C-4'), 128.38 (C-5'), 127.98 (meta-Ar), 119.66 (C-3), 63.82 (C-1), 61.98 (C-1'), 51.51 (CO_2CH_3), 18.07 (C-6'); MS (EI) calcd for $C_{23}H_{26}O_4Si$ 394.1600, found 394.1601 (M^+, 93%); Anal. Calcd for $C_{23}H_{26}O_4Si$: C, 70.0; H, 6.64. Found: C, 69.79; H, 6.99.

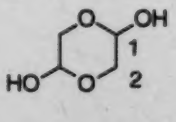


***cis*-(*E*,*E*)-2,4-Hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60).** A solution of the acid **65** (1 g, 4 mmol), DCC (0.82 g, 4 mmol), and DMAP (0.02 g, 0.16 mmol) in a mixture of diethyl ether (15 mL) and dichloromethane (5 mL) was stirred at r.t. for a few minutes. A solution of methyl (*E*)-4-hydroxy-2-butenoate (**64**) (0.47 g, 4 mmol) in dichloromethane (2 mL) was added and the reaction mixture was stirred overnight. The dicyclohexylurea produced was filtered off and the filtrate was washed with a saturated solution of sodium hydrogen carbonate (2 × 100 mL) and with brine (100 mL). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The product **60** was purified by silica gel flash column chromatography (75% hexanes in diethyl ether, R_f 0.14), affording the ester **60** as a colorless oil (0.82g, 60%). UV ($CHCl_3$ solution) λmax 240, 230 nm; IR ($CHCl_3$ cast) 3024 (w), 2940 (m), 1728 (s), 1666 (s), 1026 (s), 991 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 (td, 1H, *J* = 15.7, 4.6 Hz, H-2'), 6.20 (dd, 1H, *J* = 15.1, 2.0 Hz, H-3"), 6.05 (m, 1H, H-4"), 6.00 (td, 1H, *J* = 15.7, 2.0 Hz, H-3'), 5.75 (dq, *J* = 15.1, 6.2 Hz, 1H, H-2"), 5.55 (m, 1H, H-5"), 4.75 (m, 2H, H-1'), 4.55 (d, 2H, *J* = 6.2 Hz, H-1"), 3.70 (s, 3H, CO_2CH_3), 2.85 (m, 3H, H-1, H-2), 2.00 (m, 2H, H-3 or H-6), 1.80 (m, 2H, H-3 or H-6), 1.75 (d, 3H, *J* = 6.7 Hz, H-6"), 1.45 (m, 4H, H-4, H-5); ^{13}C NMR (75 MHz,

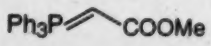
$CDCl_3$) δ 173.21 ($\underline{C}O_2R$), 173.00 ($\underline{C}O_2R$), 166.00 ($\underline{C}O_2CH_3$), 141.59 (C-2'), 134.82 (C-3"), 131.13 (C-2"), 130.46 (C-4"), 123.69 (C-5"), 121.63 (C-3'), 64.97 (C-1"), 62.50 (C-1'), 51.64 ($CO_2\underline{C}H_3$), 42.65 (C-1 and C-2), 26.43 and 26.02 (C-3 and C-6), 23.83 and 23.53 (C-4 and C-5), 18.06 (C-6"); MS (EI) calcd for $C_{19}H_{26}O_6$ 350.1729, found 350.1729 (M^+, 1), 253.1073 (M-C_6H_9O, 18), 99.0446 ($C_5H_7O_2^+$, 78), 81.0706 ($C_6H_9^+$, 100); Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.13; H, 7.48. Found: C, 65.27; 7.45.



Dihydroxymaleic acid (61). A modification of the method of Fenton[174] was used. To a solution of *D*-tartaric acid (200 g, 1.3 mol) in 100 mL of hot water was added a solution of potassium sodium tartrate (4.8 g, 17 mmol) and ferrous sulfate (4.0 g, 14 mmol) in water (100 mL). The solution was cooled to 0 °C and hydrogen peroxide (320 mL of 15% aqueous, 1.4 mol) was added over 2 hours. The mixture was stirred at 4 °C for 1 day, then cooled to 0 °C before a mixture of fuming and concentrated sulfuric acid was added slowly (80 mL of 1:3 fuming:concentrated sulfuric acid). The product **61** was allowed to crystallize at 4 °C for 3 days. The crystals were collected by vacuum filtration and washed with water. Purification by recrytallization from methanol:water 1:1 afforded dihydroxymaleic acid (**61**) as beige thin crystals (19.9 g, 10%). IR (microscope) 3516 (br s), 3439 (br s), 1940 (br m), 1635 (m), 1054 (br s) cm^{-1}; ^1H NMR (300 MHz, acetone d_6) δ 9.70 (br, $CO_2\underline{H}$), 5.00 (br, OH) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 170.64 ($\underline{C}O_2H$), 136.37 (=$\underline{C}$OH); MS (EI) calcd for $C_4H_4O_6$ 148.0008, found 148.0006 (M^+, 87), 74.0004 (M/2, 100).



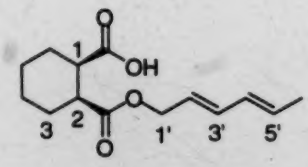
Glycolaldehyde (62). Glycolaldehyde (**62**) was prepared as reported before by Fenton.[175] Dihydroxymaleic acid (**61**) (1.45 g, 9.7 mmol) was dissolved in pyridine (7.2 mL, 89 mmol) and heated at 55 °C until gas evolution ceased (ca. 20 min). The pyridine was evaporated *in vacuo*. The resulting solid was left in a dessicator over sodium hydroxide and concentrated sulfuric acid for one week, affording glycolaldehyde (**62**) as a redish white solid mostly in the dimer form (0.6 g, 50%, ~99% dimer). The NMR data reported are for the dimer only. IR (microscope) 3409 (br s), 2971 (m), 2954 (m), 1269 (s), 1138 (s), 1099 (s), 1078 (s), 899 (s), 870 (s), 811 (s) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 5.10 (t, 2H, J = 5.1 Hz, H-1), 3.55 (d, 4H, J = 5.1 Hz, H-2); ^{13}C NMR (75 MHz, D_2O) δ 89.78 (C-1), 64.57 (C-2); MS (EI) calcd for dimer $C_4H_7O_3$ 103.0395, found 103.0399 (M-OH, 5), 102.0327 (M-H_2O, 7), 89.0256 (M-OCH_3, 89), 61.0280 (monomerH^+, 100); Anal. Calcd for $C_4H_8O_4$: C, 40.0; H, 6.71. Found: C, 40.51; H, 6.57.



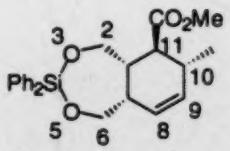
(Carbomethoxymethylene)triphenylphosphorane (63). The known compound **63**[176] was prepared as follows. To a solution of methyl bromoacetate (15 g, 98 mmol) in benzene (80 mL) was added a solution of triphenylphosphine (31 g, 120 mmol) in benzene (160 mL) dropwise. The mixture was heated under reflux overnight. The solvent was evaporated *in vacuo* and the unreacted triphenylphosphine was precipitated in water and filtered off. An aqueous solution of sodium hydroxide (4.8 g in 150 mL) was added over 40 min. The white precipitate was collected by filtration, washed with water and dried under vacuum overnight. The solid was recrystallized from ethyl acetate to afford compound **63** as white crystals (16 g, 49%). IR ($CHCl_3$ cast) 3056 (w), 2941 (w), 1619 (s), 1435 (s), 1347 (s), 1125 (s), 716 (s), 693 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.55 (m, 3H, para-Ar), 7.65 (m, 6H, meta-Ar), 7.45 (m, 6H,

ortho-Ar), 3.50 (s, 3H, $CO_2C\underline{H}_3$), 2.90 (s, 1H, $C\underline{H}CO_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.55 ($\underline{C}O_2CH_3$), 133.22, 133.05, and 132.92 (meta-Ar), 131.93 and 131.90 (para-Ar), 129.04, 128.80, 128.64, 127.31 (*ortho*-Ar), 127.98 (d, J = 99.2 Hz, Ar$\underline{C}$P), 49.77 ($CO_2C\underline{H}_3$), 29.76 (d, J = 128.0 Hz, $\underline{C}HCO_2CH_3$); MS (EI) calcd for $C_{21}H_{19}O_2P$ 334.1123, found 334.1104 (M^+, 58), 333.1043 (M-H, 100); Anal. Calcd for $C_{21}H_{19}O_2P$: C, 75.42; H, 5.73. Found: C, 75.28; H, 5.71.

Methyl (*E*)-4-hydroxy-2-butenoate (64). The method of Ainsworth *et al.*[52a] was used. To a suspension of glycolaldehyde (**62**) (2.5 g, 20.8 mmol) in benzene (375 mL) was added a solution of (carbomethoxymethylene)triphenylphosphorane (**63**) (13.9 g, 41.6 mmol) in benzene (250 mL). The mixture was heated under reflux for 4 hours. The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (75% diethyl ether in petroleum ether, R_f 0.31) to give compound **64** as a colorless oil (4.3 g, 90%). IR ($CHCl_3$ cast) 3429 (br m), 2954 (m), 1724 (s), 1707 (s), 1280 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (td, 1H, J = 15.7, 4.0 Hz, H-3), 6.10 (td, 1H, J = 15.7, 2.1 Hz, H-2), 4.35 (m, 2H, H-4), 3.75 (s, 3H, $CO_2C\underline{H}_3$), 1.85 (br s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5 ($\underline{C}O_2CH_3$), 147.09 (C-3), 119.78 (C-2), 61.83 (C-4), 51.61 ($CO_2\underline{C}H_3$); MS (EI) calcd for $C_5H_8O_3$ 116.0474, found 116.0458 (M^+, 4), 115.0395 (M-H, 26), 87.0439 (M-CHO, 46); Anal. Calcd for $C_5H_8O_3$: C, 51.70; H, 6.95. Found: C, 51.88; H, 7.39.

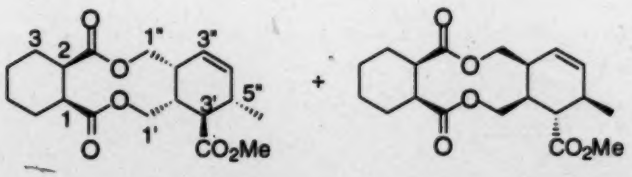


***cis*-(*E*,*E*)-2,4-Hexadien-1-yl cyclohexane-2-carboxylate carboxylic acid (65).** The procedure of Craig *et al.* was followed.[52b] A solution of *cis*-hexahydrophthalic anhydride (10.3 g, 65 mmol), (*E*,*E*)-2,4-hexadien-1-ol (7.4 mL, 65 mmol), DMAP (0.3 g, 2.6 mmol), and pyridine (7.8 mL, 96 mmol) in dichloromethane (220 mL) was heated under reflux for 18 hours. The solvent was evaporated *in vacuo*, the residue was redissolved in diethyl ether (260 mL) and extracted with 10% sodium hydroxide (2 × 100 mL). All the aqueous layers (three layer had formed) were acidified to pH 2 (2N HCl) and extracted with diethyl ether (4 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated *in vacuo* to afford the acid **65** as a colorless oil (13.2 g, 81%). IR ($CHCl_3$ cast) 2858 (br m), 1732 (s), 1704 (s), 1177 (s) cm^{-1}; ^1H NMR (acetone-d_6) δ 6.25 (dd, 1H, J = 15.0, 10.0 Hz, H-3'), 6.05 (dt, 1H, J = 10.0, 2.0 Hz, H-4'), 5.75 (dq, 1H, J = 15.0, 7.0 Hz, H-2'), 5.65 (m, 1H, H-5'), 4.55 (m, 2H, H-1'), 2.85 (m, 2H, H-1 and H-2), 2.00 (m, 2H, H-3 or H-6), 1.80 (m, 2H, H-3 or H-6), 1.75 (d, 3H, J = 7.0 Hz, H-6'), 1.45 (m, 4H, H-4, H-5); ^{13}C NMR (75 MHz, acetone-d_6) δ 174.91 ($\underline{C}O_2H$), 173.66 ($\underline{C}O_2R$), 134.64 (C-3'), 131.68 (C-2'), 131.01 (C-4'), 125.41 (C-5'), 64.90 (C-1'), 43.10 (C-2), 42.80 (C-1), 29.02, 27.05, 26.88, 24.45 (C-3, C-4, C-5, C-6), 18.08 (C-6'); MS (EI) calcd for $C_{14}H_{20}O_4$ 252.1362 (M^+, 21), 109.0653 ($C_7H_9O^+$, 50), 155.0713 (M-C_6H_9O, 41), 81.0710 ($C_6H_9^+$, 100); Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.53; H, 7.96. Found: C, 66.53; H, 7.95.



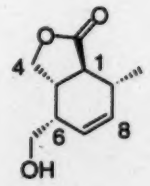
[1*R,7*R**,10*S**,11*R**]-11-carbomethoxy-10-methyl-4,4-diphenyl-3,5,4-dioxasilabicyclo[5.4.0]-8-undecene (66).** The Diels-Alder reaction of triene **59** was

performed using the method of Ainsworth *et al.*[52a] The ester **59** (0.15 g, 0.38 mmol) was azeotropically dried by evaporation *in vacuo* with toluene (3 × 4 mL). The dried ester **59** was redissolved in toluene (6.3 mL), three times sonicated for 5 min, and degassed with Ar for 5 min. The solution was then gently transferred into a thick-walled resealable tube and heated at 180 °C for 5 days, then at 160 °C for 8 days. The toluene was evaporated *in vacuo* and the sample was left under high vacuum for 1 day to afford the product as an oil (0.16 g, quant.). The structure of **66** was confirmed by cleavage of the silyl tether as described below. IR ($CHCl_3$ cast) 3018 (w), 2874 (w), 1732 (s), 1430 (m), 1117 (s), 1054 (s), 720 (s), 700 (s), 515 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 (m, 4H, *ortho*-Ar), 7.40 (m, 6H, meta and para-Ar), 5.55 (m, 2H, H-8, H-9), 4.15 (dd, 1H, J = 12.3, 1.4 Hz, H_a-2), 3.95 (dd, 1H, J = 15.3, 3.8 Hz, H_b-2), 3.85 (s, 3H, $CO_2C\underline{H}_3$), 3.75 (d, 2H, J = 6.4 Hz, H-6), 2.70 (m, 1H, H-1), 2.55 (d, 1H, J = 6.4 Hz, H-7), 2.35 (s, 1H, H-11), 2.25 (m, 1H, H-10), 1.00 (d, 3H, J = 6.5 Hz, 10-Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.91 ($\underline{C}O_2CH_3$), 135.00 and 134.78 (*ortho*-Ar), 134.06 (C-8), 130.58 (meta-Ar), 128.23 (Ar$\underline{C}$-Si), 127.91 (para-Ar), 124.98 (C-9), 63.77 (C-2), 63.55 (C-6), 51.70 ($CO_2\underline{C}H_3$), 46.05, 42.04, 41.39, 35.83 (C-1, C-7, C-10, C-11), 20.08 (10-$\underline{C}H_3$); MS (EI) calcd for $C_{23}H_{26}O_4Si$ 394.1600, found 394.1597 (M^+, 16), 364.1502 (M-CH_2O, 100), 316.1134 (M-C_6H_6, 59), Anal. Calcd for $C_{23}H_{26}O_4Si$: C, 70.02; H, 6.64. Found C, 70.00; H, 6.35.

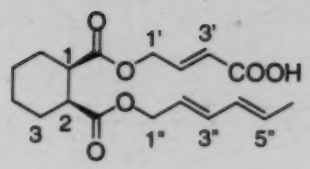


Diels-Alder of *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3'-carbomethoxy-2'-propen-1-yl cyclohexane-1,2-dicarboxylate (60) affording the mixture of diastereoisomers

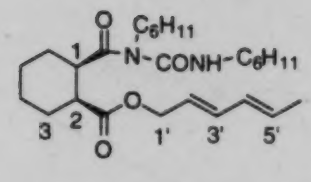
67. The procedure of Craig *et al.* was followed.[52b] The ester **60** (50 mg, 142 μmol) was azeotropically dried by evaporation *in vacuo* with toluene (3 × 6 mL), redissolved in toluene (7 mL), sonicated three times for 5 min, and degassed with Ar for 5 min. The solution was then gently transferred into a thick-walled resealable tube and heated at 170 °C for 10 days. The solvent was concentrated *in vacuo* and the products **67** were purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, R_f 0.38 with 33%) affording a white solid (12.3 mg, 40%). The starting material (R_f 0.33 with 33% ethyl acetate in hexanes) could be recovered and recycled (28.3 mg, 55%). $[\alpha]_D^{25}$ -2.4 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$ cast) 2950 (w), 1734 (s), 1231 (m), 1165 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.60 (m, 2H, H-3", H-4"), 4.30 (d, 1H, *J* = 11.9 Hz, H_a-1'), 4.00 (d, 2H, *J* = 8.7 Hz, H-1"), 3.75 (s, 3H, $CO_2C\underline{H}_3$), 3.65 (t, 1H, *J* = 10.4 Hz, H_b-1'), 3.10 (m, 1H, H-2', H-2", H-3', or H-5"), 2.95 (m, 1H, H-2', H-2", H-3', or H-5"), 2.65 (m, 1H, H-2', H-2", H-3', or H-5"), 2.50 (m, 2H, H-1, H-2), 2.10 (m, 1H, H-2', H-2", H-3', or H-5"), 2.09 (m, 1H), 1.95 (m, 1H), 1.80 (m, 1H), 1.65 (m, 2H), 1.45 (m, 2H), 1.25 (m, 1H), 0.95 (d, 3H, *J* = 7.1 Hz, H-6"); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.13, 174.361, 173.86 (3 $\underline{C}O_2R$), 134.40 (C-3" or C-4"), 124.32 (C-3" or C-4"), 65.20 (C-1' or C-1"), 63.71 (C-1' or C-1"), 51.91 ($CO_2\underline{C}H_3$), 46.54 (C-1, C-2), 42.20, 37.57, 35.04, 33.72, 31.00, 26.32, 24.37, 21.09 (C-3, C-4, C-5, C-6, C-2', C-3', C2", C-5"), 19.74 (C-6"); MS (EI) calcd for $C_{19}H_{26}O_6$ 350.1729, found 350.1726 (M^+, 1), 172.0990 ($C_8H_{12}O_4^+$, 58), 119.0858 ($C_9H_{11}^+$, 100); Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.11; H, 7.48. Found: C, 64.87; H, 7.55.



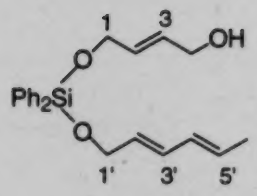
6-(Hydroxymethyl)-9-methyl-2-oxo-3-oxabicyclo[3.4.0]-7-nonene (68). The Diels-Alder reaction of compound **59** and cleavage of the silyl tether was performed using the method of Ainsworth *et al.*[52a] The ester **59** (0.34 g, 0.86 mmol) was azeotropically dried by evaporation *in vacuo* with toluene (3 × 10 mL). The dried ester **59** was redissolved in toluene (14.4 mL), sonicated three times for 5 min and degassed with Ar for 5 min. The solution was then gently transferred into a thick-walled resealable tube and heated at 160 °C for 10 days. The solvent was concentrated *in vacuo* and the residue was redissolved in methanol (14.5 mL). A 48% HF (2.5 mL) solution was added and the mixture was stirred at r.t. for 7 hours. The mixture was neutralized with sodium hydrogen carbonate, diluted with water, and extracted with diethyl ether (5 × 50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated *in vacuo*. The product **68** was purified by silica gel flash column chromatography (50 to 60% diethyl ether in petroleum ether, R_f 0.32 with 100%), affording the product as a white solid (68 mg, 44%, d.e. > 95%). $[\alpha]_D^{25}$ –2.4 (*c* 0.5, $CHCl_3$); UV λmax (isopropyl ether) 240 nm; IR ($CHCl_3$ cast) 3442 (br m), 3015 (w), 2959 (w), 2916 (m), 2876 (m), 1776 (s), 1372 (w), 1184 (s), 1090 (s), 1058 (s), 993 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.65 (td, 1H, *J* = 9.9, 1.7 Hz, H-8), 5.55 (ddd, 1H, *J* = 9.9, 4.6, 2.4 Hz, H-7), 4.40 (dd, 1H, *J* = 9.0, 7.0 Hz, H-4 down), 4.30 (dd, 1H, *J* = 11.7, 9.0 Hz, H-4 up), 3.70 (dd, 1H, *J* = 11.2, 3.7 Hz, C$\underline{H}_2$OH up), 3.60 (dd, 1H, *J* = 11.2, 7.7 Hz, C$\underline{H}_2$OH down), 2.60 (m, 1H, H-6), 2.50 (m, 1H, H-5), 2.40 (m, 1H, H-9), 2.10 (dd, 1H, *J* = 14.1, 10.4 Hz, H-1), 1.80 (br s, 1H, OH), 1.20 (d, 3H, *J* = 7.0 Hz, 9-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.45 (C-2), 136.82 (C-8), 125.60 (C-7), 69.81 (C-4), 61.83 ($\underline{C}H_2OH$), 42.97 (C-1), 41.18 (C-5), 38.83 (C-6), 32.37 (C-9), 18.90 (9-CH_3); MS (EI) calcd for $C_{10}H_{14}O_3$ 182.0943, found 182.0948 (M^+, 3), 164.0840 (M-H_2O, 87), 91.0549 ($PhCH_2^+$, 100); Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.64; H, 7.75.



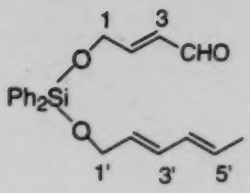
***cis*-(*E*,*E*)-2,4-Hexadien-1-yl-3-carboxy-(*E*)-2-propen-1-yl cyclohexane-1,2-dicarboxylate (73).** A solution of the ester **60** (0.52 g, 1.48 mmol) and lithium hydroxide monohydrate (1.6 mL of a 1 M aqueous solution) in a mixture of water (5 mL) and THF (15 mL) was stirred at r.t. for one day. The THF was evaporated *in vacuo* and the resulting aqueous layer was acidified to pH 1 and extracted with chloroform (4 × 50 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*, affording the crude acid **73** (0.59 g, 95%). The acid **73** was purified by silica gel flash column chromatography (10 to 40%, then 100% diethyl ether in petroleum ether, R_f 0.21 with 100%) affording a beige semi-solid (191 mg, 50%). IR ($CHCl_3$ cast) 3024 (br m), 2937 (s), 1731 (s), 1701 (s), 1662 (m), 1303 (m), 1245 (m), 1175 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (td, 1H, *J* = 15.7, 4.3 Hz, H-2'), 6.25 (dd, 1H, *J* = 15.2, 10.5 Hz, H-3"), 6.05 (m, 2H, H-3' and H-4"), 5.75 (dq, 1H, *J* = 15.2, 6.7 Hz, H-2"), 5.55 (m, 1H, H-5"), 4.75 (dd, 2H, *J* = 4.3, 1.9 Hz, H-1'), 4.55 (d, 2H, *J* = 6.7 Hz, H-1"), 2.95-2.75 (m, 2H, H-1, H-2), 2.05 (m, 2H, H-3 or H-6) 1.85 (m, 2H, H-3 or H-6), 1.75 (d, 3H, *J* = 6.5 Hz, H-6"), 1.60-1.30 (m, 4H, H-4, H-5); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.28, 173.04 (CO_2R), 170.88 (CO_2H), 144.09 (C-2'), 134.93 (C-3"), 131.24 (C-2"), 130.44 (C-4"), 123.60 (C-5"), 121.08 (C-3'), 65.07 (C-1"), 62.38 (C-1'), 42.65 (C-1, C-2), 26.46, 26.00 (C-3, C-6), 23.84, 23.51 (C-4, C-5), 18.09 (C-6"); MS (EI) calcd for $C_{18}H_{24}O_6$ 336.1573, found 336.1572 (M^+, 1), 239.0922 (M-C_6H_9O, 37), 97.0654 ($C_6H_9O^+$, 46), 81.0704 ($C_6H_9^+$, 100); Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.26; H, 7.20. Found: C, 63.65; H, 7.39.



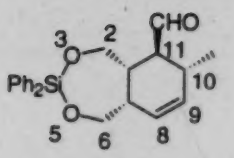
***N*-cyclohexyl-*N*-[*cis*-(*E*,*E*)-2,4-hexadien-1-yl cyclohexane-2-carboxylate-1-carbonyl]-*N'*-cyclohexylurea (75).** To a solution of the acid **73** (0.1 g, 0.3 mmol) in dichloromethane (2 mL) at -20°C were added simultaneously a solution of *N*-acetylcysteamine (0.04 mL, 0.35 mmol) in dichloromethane (1.5 mL) and a solution of DCC (0.075 g, 0.36 mmol) and DMAP (9 mg, 0.02 mmol) in dichloromethane (3 mL). The mixture was stirred at r.t. overnight, after which the solvent was evaporated *in vacuo*. Purification by silica gel flash chromatography (25% diethyl ether in petroleum ether, R_f 0.75 with 50%) afforded compound **65** (50 mg, 50%) and compound **75** as a yellow oil (25 mg, 20%). The latter was found by ^{1}H NMR to be unstable. IR ($CHCl_3$ cast) 3341 (br m), 2930 (s), 1740 (s), 1702 (s), 1657 (s), 1217 (s) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.60 (br s, 1H, NH), 6.25 (dd, 1H, *J* = 15.0, 10.5 Hz, H-3'), 6.05 (ddd, 1H *J* = 15.0, 10.5, 1.5 Hz, H-4'), 5.75 (m, 1H, H-2'), 5.58 (dt, 1H, *J* = 15.0, 6.6 Hz, H-5'), 4.62 (dd, 1H, *J* = 13.1, 7.1 Hz, H_a-1'), 4.50 (dd, 1H, *J* =13.1, 6.7 Hz, H_b-1'), 4.10 (tt, 1H, *J* = 10.9, 3.6 Hz, NC<u>H</u>), 3.65 (m, 1H, NHC<u>H</u>), 2.45 (td, 1H, *J* = 12.5, 4.5 Hz, H-1 or H-2), 2.38 (dt, 1H, *J* = 12.5, 4.0 Hz, H-1 or H-2), 1.75 (d, 3H, *J* = 6.6 Hz, H-6'), 2.0-1.1 (m, 28H, H-3, H-4, H-5, H-6, 10 C<u>H</u>$_2$ from *N*-cyclohexyls); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.65 (CO_2R), 171.80 (R<u>C</u>ONR), 153.00 (N<u>C</u>ONHR), 133.64 (C-3'), 130.00 (C-2'), 128.91 (C-4'), 122.03 (C-5'), 63.76 (C-1'), 52.31, 48.43 (N<u>C</u>H, NH<u>C</u>H), 42.77, 41.26 (C-1, C-2), 31.64, 31.38, 30.54, 30.17, 28.45, 26.95, 24.62, 24.60, 23.96, 23.76, 23.40, 23.20, 20.87, 19.27 (C-3, C-4, C-5, C-6, 10 <u>C</u>H$_2$ from *N*-cyclohexyls), 16.66 (C-6'); MS (ES) 481 (MNa^+, 58), 459 (MH^+, 65), 379 (M-C_6H_8, 100); MS (EI) calcd for $C_{27}H_{42}N_2O_4$ 458.3144, found 458.3151 (M^+, 10), 236.1652 (M-$C_{13}H_{20}NO_2$, 52), 81.0706 ($C_6H_9^+$, 100).



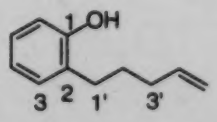
[4-Hydroxy-(*E*)-2-buten-1-oxy]-[(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (78). To a solution of the ester **59** (0.87 g, 2.2 mmol) in dichloromethane (5.3 mL) cooled to –65 °C was added a solution of DIBAL-H (1.1 mL, 6.2 mmol) in dichloromethane (3 mL) over 20 min. The mixture was stirred at –65 °C for 2.5 hours, quenched by dropwise addition of diethyl ether:methanol 2:1 (6 mL). The reation mixture was warmed to r.t., diluted with diethyl ether (150 mL), washed with a solution of saturated potassium sodium tartrate (4 × 75 mL), brine (75 mL), then dried ($MgSO_4$) and evaporated *in vacuo*. The residue was left under high vacuum overnight to afford the alcohol **78** (0.4 g, 50%). UV λmax (isopropyl ether solution) 240 nm; IR ($CHCl_3$ cast) 3374 (m), 3070 (m), 2856 (m), 1430 (m), 1125 (s), 1117 (s), 989 (s), 719 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.65 (dd, 4H, *J* = 7.8, 1.4 Hz, *ortho*-Ar), 7.40 (m, 6H, meta-Ar, para-Ar), 6.20 (dd, 1H, *J* = 15.0, 10.4 Hz, H-3'), 6.05 (m, 1H, H-4'), 5.95-5.75 (m, 2H, H-3, H-2'), 5.75-5.60 (m, 2H, H-2, H-5'), 4.34 (d, 2H *J* = 4.8 Hz, H-1 or H-1'), 4.34 (d, 2H, *J* = 4.6 Hz, H-1 or H-1'), 4.13 (m, 2H, H-4), 1.75 (d, 3H, *J* = 6.8 Hz, H-6'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.92 (*ortho*-Ar), 132.58 (Ar<u>C</u>-Si), 131.15 (C-3'), 130.87 (C-4'), 130.37 (C-2), 130.04 (para-Ar), 129.83 (C-3), 129.58 (C-2'), 128.69 (C-5'), 127.87 (meta-Ar), 63.66 and 63.10 (C-1, C-1', C-4), 18.08 (C-6'); MS (EI) calcd for $C_{22}H_{26}O_3Si$ 366.1651, found 366.1639 (M^+, 4), 199.9579 (M-$C_5H_6O_3$, 100); Anal. Calcd for $C_{22}H_{26}O_3Si$: C, 72.09; H, 7.15. Found: C, 72.19; H, 7.22.



[3-Carboxyaldehyde-(*E*)-2-propen-1-oxy]-[(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (79). The general procedure for the Swern oxidation was used.[45] A 0.1 M solution of oxalyl chloride (0.36 mL, 0.36 mmol) was cooled in a dry ice-acetone bath, then DMSO (50 μL, 0.72 mmol) was slowly added and the mixture was stirred for 7 min. A solution of the alcohol **78** (0.12 g, 0.33 mmol) in dichloromethane (0.5 mL) was added at -70 °C over 10 min. The mixture was stirred at that temperature for 30 min. Triethylamine (0.14 mL, 0.98 mmol) was added over 10 min, and the mixture was stirred for 20 min in the bath, then for 30 min outside the bath. The solution was diluted with one volume of diethyl ether, washed with water (5 × 5 mL), with brine (2 × 5 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The aldehyde **79** could not be fully purified because of its instability (41 mg crude, 35%). Purification by Florisil column chromatography (0-30% diethyl ether in petroleum ether, R_f 0.83 and 0.37) afforded a mixture of aldehyde **79** and its Diels-Alder adduct **80** in a 75:25 ratio respectively. The mixture was then used without further purification in the Diels-Alder reaction. The data reported here are for compound **79**. ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.60 (d, 1H, J = 8.0 Hz, CHO), 7.70-7.30 (m, 10H, Ar), 6.85 (td, 1H, J = 15.5, 3.4 Hz, H-2), 6.50 (tdd, 1H, J = 15.5, 8.0, 2.0 Hz, H-3), 6.20 (dd, 1H, J = 15.0, 10.4 Hz, H-3'), 6.05 (br t, 1H, J = 10.4 Hz, H-4'), 5.65 (m, 1H, H-2'), 5.55 (m, 1H, H-5'), 4.60 (dd, 2H, J = 3.4, 2.0 Hz, H-1), 4.35 (d, 2H, J = 5.4 Hz, H-1'), 1.70 (t, 3H, J = 6.8 Hz, H-6'); MS (CI, NH_3) 382 (MNH_4^+, 20), 365 (MH^+, 100).

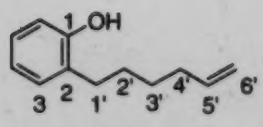


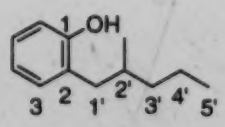
[1*R,7*R**,10*S**,11*R**]-11-Carboxyaldehyde-10-methyl-4,4-diphenyl-3,5,4-dioxasilabicyclo[5.4.0]-8-undecene (80).** An Ar degassed solution of the crude aldehyde **79** (3 mg, 8.2 μmol) in toluene (0.2 mL) was placed in a thick-walled resealable tube and heated at 160 °C for 34 hours. Evaporation of the toluene *in vacuo* afforded the Diels-Alder adduct **79** (1.9 mg, 60%, d.e. > 90%). IR ($CHCl_3$ cast) 3050 (w), 2928 (m), 1727 (s), 1127 (s), 1118 (s), 1074 (s), 699 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 10.05 (s, 1H, CHO), 7.7-7.2 (m, 10H, Ar), 5.5 (m, 2H, H-8, H-9), 4.20 (m, 2H, H_a-2), 3.80 (m, 2H, H-6), 2.65 (m, 3H, H-1, H_b-2, H-7), 2.30 (m, 1H, H-10), 2.20 (2, 1H, H-11), 0.95 (d, 3H, J = 6.5 Hz, 10-Me); MS (CI, NH_3) 382 (MNH_4^+, 30), 365 (MH^+, 100).

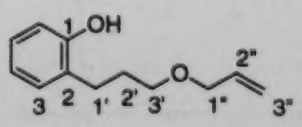


2-(4-Penten-1-yl)phenol (82). A modification of the procedure of Yates and Macas was used.[142] To a 50% oil dispersion of sodium hydride (0.82 g, 17 mmol) washed with hexane (2 × 10 mL) and with diethyl ether (10 mL) was added DMSO (8.1 mL, 114 mmol). The mixture was heated at 80 °C until gas evolution ceased. Once the

reaction mixture was cooled to r.t., a solution of methyltriphenyl phosphonium bromide (6.1 g, 17 mmol) in DMSO (30 mL) was added. The mixture was stirred at r.t. for 20 min before a solution of the hemiacetal **86** (1.0 g, 8.6 mmol) in DMSO (2.2 mL) was added. The mixture was stirred at r.t. for 50 min. The reaction was quenched by addition of water (7.5 mL) followed by acidification to pH 5 (1N HCl). The mixture was extracted with diethyl ether (4 × 50 mL), and the combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 30% diethyl ether in petroleum ether, R_f 0.68 with 50%) to afford the phenol **82** as a yellow oil (0.56 g, 40% over 2 steps). IR ($CHCl_3$ cast) 3448 (br m), 2928 (m), 1591 (m), 1502 (m), 1489 (m), 1454 (m), 1234 (s), 1203 (s), 911 (s), 751 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, 1H, J = 7.9, 1.5 Hz, H-3), 7.10 (dt, 1H, J = 7.9, 1.5 Hz, H-5), 6.9 (dt, 1H, J = 7.9, 1.2 Hz, H-4), 6.8 (dd, 1H, J = 7.9, 1.2 Hz, H-6), 5.9 (m, 1H, H-4'), 5.0 (m, 2H, H-5'), 2.6 (t, 2H, J = 7.5 Hz, H-1'), 2.2 (tq, 2H, J = 6.2, 1.3 Hz, H-3'), 1.75 (m, 2H, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.46 (C-1), 138.70 (C-3), 130.29 (C-5), 128.27 (C-2), 127.14 (C-4), 120.82 (C-6), 115.27 (C-4'), 114.85 (C-5'), 33.40 (C-1'), 29.27 (C-3'), 28.89 (C-2'); MS (EI) calcd for $C_{11}H_{14}O$ 162.1045, found 162.1038 (M^+, 31), 120.0569 (M-C_3H_6, 58), 107.0495 (M-C_4H_7, 100), 91.055 ($C_7H_7^+$, 16), 77.039 ($C_6H_5^+$, 25); Anal. Calcd for $C_{11}H_{14}O$: C, 81.44; H, 8.70. Found: C, 80.05; H, 9.11.







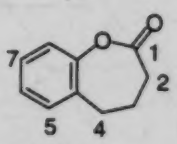
2-(5-Hexen-1-yl)phenol (83), 2-(2-methyl-4-penten-1-yl)phenol (88) and 2-(3-allylhydroxybutyl)phenol (89). The procedure of Richey and Domalski was used.[143] To a suspension of magnesium turnings (1.5 g, 63 mmol) in diethyl ether (20

mL) was added a solution of allyl bromide (5.2 mL, 60.5 mmol) in diethyl ether (15 mL). The solution was heated under reflux for 30 min. While refluxing, a solution of 2-allylphenol (2.0 g, 14.9 mmol) in diethyl ether (10 mL) was added. The mixture was heated under reflux until the gas evolution ceased, and then transferred into a resealable tube and heated at 100 °C for 4 hours. Once at r.t., the mixture was poured into 150 mL of 1N HCl and extracted with diethyl ether (3 × 150 mL). The combined ethereal layers were extracted with 5% sodium hydroxide (3 × 300 mL). The combined basic layers were acidified to pH 5 (1N HCl) and extracted with diethyl ether (4 × 500 mL). The latest organic layers were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5 to 20% diethyl ether in petroleum ether, R_f 0.67 with 50%) to give a mixture of 2-(5-hexenyl)phenol (**83**) and 2-(2-methyl-4-penten-1-yl)phenol (**88**) in a 3:1 ratio (total 0.68 g, 30%). Allyl 2-(3-allylhydroxybutyl)phenol (**89**) was also isolated (R_f 0.36, 0.07 g, 5%).

Data for 2-(5-hexenyl)phenol (83). IR ($CHCl_3$ cast) 3450 (br m), 2858 (m), 1455 (s), 1232 (m), 1170 (m), 911 (m), 751 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (m, 2H, H-3, H-5), 6.85 (dt, 1H, J = 7.4, 1.0 Hz, H-4), 6.75 (dd, 1H, J = 7.9, 1.0 Hz, H-6), 5.85 (m, 1H, H-5'), 5.00 (m, 2H, H-6'), 2.65 (t, 2H, J = 7.5 Hz, H-1'), 2.10 (q, 2H, J = 6.4 Hz, H-4'), 1.65 (m, 2H, H-2'), 1.45 (m, 2H, H-3'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.38 (C-1), 138.94 (C-3), 131.30 (C-5), 130.21 (C-2), 127.07 (C-4), 120.82 (C-6), 115.21 (C-5'), 114.45 (C-6'), 33.58 (C-1'), 29.75 (C-4'), 29.21 (C-3'), 28.70 (C-2'); MS (EI) calcd for $C_{12}H_{16}O$ 176.1201, found 176.1199 (M^+, 42), 147.0806 (M-Et, 4), 133.0651 (M-Pr, 24), 120.0574 (M-C_4H_8, 28), 107.0493 (M-C_5H_9, 100), 77.0395 ($C_6H_5^+$, 20).

Data for 2-(3-allylhydroxybutyl)phenol (89). IR ($CHCl_3$ cast) 3316 (br s), 2921 (s), 1489 (s), 1457 (s), 1245 (s), 754 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.15 (dt, 1H, J = 8.0, 1.8 Hz, H-5), 7.05 (dd, 1H, J = 7.4, 1.8 Hz, H-3), 6.95 (dd, 1H, J = 8.0,

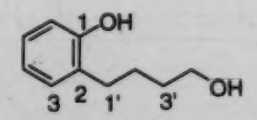
1.2 Hz, H-6), 6.85 (dt, 1H, *J* = 7.4, 1.2 Hz, H-4), 5.83 (m, 1H, H-2"), 5.10 (ddd, 1H, *J* = 17.1, 3.2, 1.7 Hz, H-3"*trans*), 5.02 (ddd, 1H, *J* = 10.2, 3.2, 1.2 Hz, H-3"*cis*), 4.04 (m, 2H, H-1"), 2.83 (m, 2H, H-3'), 2.20 (m, 2H, H-1'), 1.63 (m, 2H, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.57 (C-1), 138.00 (C-3), 131.58 (C-5), 128.39 (C-4), 125.22 (C-2), 120.32 (C-6), 117.23 (C-2"), 115.63 (C-3"), 74.18 (C-1"), 39.11 (C-3'), 35.69 (C-1'), 30.25 (C-2'); MS (EI) calcd for $C_{12}H_{16}O_2$ 192.1135, found 192.1135 (M^+, 30), 108.0578 ($C_7H_8O^+$, 100); Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.62; H, 8.94. Found: C, 74.98; H, 8.96.



***o*-4-Butanalphenol ε-lactone (84).** The procedure for this Baeyer-Villiger reaction was based on the work of Emmons and Lucas.[177] Peroxitrifluoroacetic acid was prepared by the slow addition of trifluoroacetic anhydride (20 mL, 133 mmol) to a solution of 90% aqueous hydrogen peroxide (3 mL, 112 mmol) in dichloromethane (20 mL). This solution was then added to a suspension of α-tetralone (5 mL, 37 mmol) and Na_2HPO_4 (48 g, 339 mmol) in dichloromethane (80 mL) over 30 min. The solution came to reflux during the addition, and was maintained at reflux for 4 hours. The precipitate that formed was filtered and rinsed with dichloromethane. The filtrate was washed with a 5% solution of sodium carbonate (4 × 200 mL). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*, affording the product **84** as a yellow oil which was used without further purification (5.7 g, 93%). IR ($CDCl_3$ cast) 2849 (w), 2868 (w), 1766 (s), 1607 (w), 1215 (s), 1095 (s), 760 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.25 (ddd, 1H, *J* = 7.9, 7.4, 2.1 Hz, H-7), 7.20 (dt, 1H, *J* = 7.4, 2.1 Hz, H-5), 7.15 (dt, *J* = 7.4, 0.9 Hz, H-6), 7.10 (br d, 1H, *J* = 7.9 Hz, H-8), 2.80 (t, 2H, *J* = 7.3 Hz, H-2), 2.50 (t, 2H, *J* = 7.3 Hz, H-4), 2.20 (p, 2H, *J* = 7.3 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ

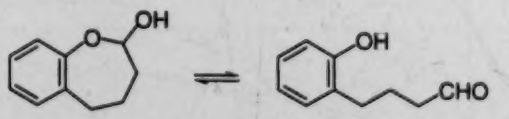
171.68 (C-1), 151.93 (C-8a), 130.174 (C-8), 129.77 (C-4a), 128.41 (C-6), 126.00 (C-5), 119.39 (C-7), 31.18 (C-2), 28.34 (C-4), 26.64 (C-3); MS (EI) calcd for $C_{10}H_{10}O_2$ 162.0681, found 162.0687 (M^+, 40), 134.0736 (M-CO, 5), 117.0708 (M-HCO_2H, 17), 107.0499 ($C_7H_7O^+$, 100); Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. Found: C, 73.68; H, 6.39.

Alternative procedure for the preparation of 84. A modification of the method of Crimmins *et al.*[178] was used. Commercial MCPBA was washed with phosphate buffer (400 mL of 0.5 M Na_2HPO_4) and dried under high vacuum for one day. A solution of washed MCPBA (16 g, 94 mmol) in dichloromethane (17 mL) was added to a solution of α-tetralone (5.5 g, 37.6 mmol) in dichloromethane (60 mL), and the mixture was heated at 40 °C for two days. The precipitate that formed was filtered and washed with 10% sodium carbonate. The organic layer was poured into 10% sodium thiosulfate (400 mL) and stirred vigorously. Sodium hydrogen carbonate was added (ca. 100 mL) and the aqueous layer was extracted with dichloromethane (200 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. Purification of the residue by silica gel column chromatography (25 % diethyl ether in petroleum ether, R_f 0.50 with 50%) afforded compound **84** which crystallized to a white solid over time (2.6 g, 50%) with identical spectroscopic data as above.

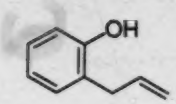


2-(4-Hydroxybut-1-yl)phenol (85). To a solution of the lactone **84** (0.56 g, 3.4 mmol) in toluene (7.7 mL) cooled to -65 °C was added a solution of DIBAL-H (2.4 mL of 1.5 M in toluene, 3.6 mmol) over 1.5 hour. The reaction mixture was stirred at -78 °C for two hours. Water was added at -65 °C (2 mL) before the solution was warmed to r.t.

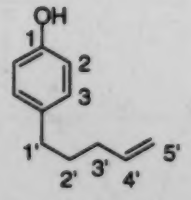
Some Celite was added and the mixture was filtered. Both the filtrate and the filter cake were extracted with diethyl ether (5 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Purification by silica gel flash column chromatography (50% diethyl ether in petroleum ether, R_f 0.11) afforded the diol **85** as a colorless oil (0.27 g, 50%). IR ($CHCl_3$ cast) 3334 (br s), 3069 (m), 3036 (m), 2938 (s), 2862 (s), 1592 (m), 1455 (s), 1239 (s), 753 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.10 (m, 2H, H-5, H-3), 6.85 (dt, 1H, J = 7.4, 1.1 Hz, H-4), 6.80 (dd, 1H, J = 7.9, 1.1 Hz, H-6), 4.10 (br s, 2H, OH), 3.70 (t, 2H, J = 6.2 Hz, H-4'), 2.60 (t, 2H, J = 7.3 Hz, H-1'), 1.70 (m, 2H, H-2'), 1.60 (m, 2H, H-3'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.88 (C-1), 130.28 (C-3), 128.39 (C-2), 127.18 (C-5), 120.56 (C-4), 115.62 (C-6), 63.12 (C-4'), 31.10 (C-1'), 29.43 (C-2'), 26.68 (C-3'); MS (EI) calcd for $C_{10}H_{14}O_2$ 166.0994, found 166.0993 (M^+, 40), 148.0885 (M-H_2O, 14), 133.0646 (M-$CH_3O^+H_2$, 27), 120.0574 (M-OEt, 25), 107.0492 (M-OPr, 100), 77.0394 ($C_6H_5^+$, 18); Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.77; H, 8.50.



***o*-4-Butanalphenol ε-hemi-acetal (86).** To a solution of the lactone **84** (1.1 g, 6.5 mmol) in toluene (20 mL) at -78 °C was added a solution of DIBAL-H (4.4 mL of 1.5 M in toluene, 6.6 mmol) over 1.5 hour. The reaction mixture was stirred at -78 °C for 2.5 hours. Water was added at -78 °C (40 mL) before the solution was warmed to r.t. Some Celite was added and the mixture was filtered. Both the filtrate and the filter cake were extracted with diethyl ether. The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and evaporated *in vacuo*, affording **86** (0.94 g, 88%). Because of its instability, the product **86** was used in the next step without purification.



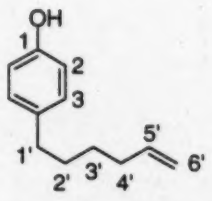
2-Allylphenol (87). The procedure of Richey and Domalski was used.[143] Allyl phenyl ether (40 mL, 0.29 mol) was heated to reflux (~ 210 °C) overnight. Once at r.t., the solution was dissolved in 5% sodium hydroxide (400 mL) and washed with diethyl ether (2 × 400 mL). The aqueous layer was then acidified to pH 5 and back extracted with diethyl ether (3 × 300 mL). The combined acidic extracts were washed with brine (600 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The product was purified by distillation, affording 2-allylphenol (**87**) as a colorless oil (23.3 g, 60%). Bp 60 °C (2 mmHg) [lit.[143] bp 53°C (0.10 mm Hg)]; IR ($CHCl_3$ cast) 3453 (br m), 3077 (w), 2978 (w), 1638 (w), 1488 (s), 1455(s), 1216 (s), 1170 (s), 839 (m), 752 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.15 (m, 2H, H-3, H-5), 6.90 (dt, 1H, J = 7.4, 1.1 Hz, H-4), 6.85 (br d, 1H, J = 8.0 Hz, H-6), 6.05 (m, 1H, H-2'), 5.20 (m, 1H, H-3'*trans*), 5.15 (m, 1H, H-3'*cis*), 5.00 (s, 1H, OH), 3.45 (d, 2H, J = 6.3 Hz, H-1'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.09 (C-1), 136.41 (C-3), 130.47 (C-5), 127.93 (C-4), 125.32 (C-2), 121.00 (C-6), 116.51 (C-2'), 115.84 (C-3'), 35.13 (C-1'); MS (EI) calcd for $C_9H_{10}O$ 134.0732, found 134.0727 (M^+, 100), 133.0646 (M-H, 43), 119.0496 ($M-CH_3$, 35), 117.0699 (M-OH, 10), 115.0547 ($M-H_3O^+$, 26), 107.0492 ($M-C_2H_3$, 17), 91.0549 ($C_7H_7^+$, 28), 77.0403 ($C_6H_6^+$, 18); Anal. Calcd for $C_9H_{10}O$: C, 80.56; H, 7.51. Found: C, 80.61; H, 7.80.



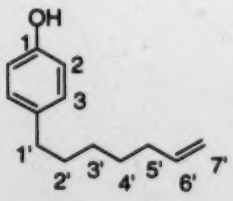
4-(Pent-4-enyl)phenol (92). Compound **117** (0.10 g, 0.57 mmol) and sodium thiomethoxide (0.4 g, 5.7 mmol) were dissolved in DMF (10 mL) and heated under reflux overnight. The brown solution was acidified to pH 1 and the methanethiol generated was evaporated by gentle heating for 30 min. The mixture was extracted with diethyl ether (4 × 20 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.57 with 50%) to give the product **92** as a colorless oil (50 mg, 55%). IR ($CHCl_3$ cast) 3341 (br s), 2975 (m), 2928 (s), 1613 (s), 1598 (m), 1514 (s), 1445 (m), 1257 (m), 1230 (s), 911 (m), 823 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-3) and 6.80 (m, 2H, H-2), 5.80 (m, 1H, H-4'), 5.05 (m, 2H, H-5'), 4.55 (br s, 1H, OH), 2.60 (t, 2H, J = 7.6 Hz, H-1'), 2.10 (dq, 2H, J = 7.6, 1.0 Hz, H-3'), 1.70 (dtt, 2H, J = 7.6, 7.6, 6.3 Hz, H-2'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.48 (C-1), 138.64 (C-4'), 134.73 (C-4), 129.49 (C-3), 115.08 (C-2), 114.62 (C-5'), 34.37 (C-1'), 33.19 (C-3'), 30.81 (C-2'); MS (EI) calcd for $C_{11}H_{14}O$ 162.1045, found 162.1044 ($M^{\bullet}$, 13), 120.0576 (M-C_3H_6, 67), 107.0498 (M-C_4H_7, 100), 91.0548 ($C_7H_7^{+}$, 11), 77.03090 ($C_6H_5^{+}$, 19).

Alternative procedure for the preparation of 92. A modification of the method of Wildes *et al.*[152] was used. A mixture of thiophenol (1.6 mL, 15.6 mmol) and sodium hydride (0.52 g of 60% oil dispersion washed with hexane (4 × 10 mL), 26 mmol) was heated under reflux for one hour. Once cooled, diethylene glycol (15 mL) was added, followed by compound **117** (0.098 g, 0.5 mmol). The mixture was heated under reflux overnight. The brown solution was diluted with water (50 mL) and washed with hexanes (3 × 80 mL). The aqueous layer was extracted with diethyl ether (3 × 80 mL), and the combined organic layers were extracted with 5% sodium hydroxide (2 × 250 mL). After acidification (pH 2, 2N HCl), the aqueous layer was extracted with diethyl ether (3 × 400 mL). The combined acidic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by silica gel flash column

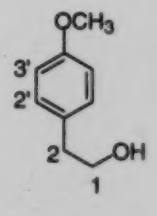
chromatography (30% diethyl ether in petroleum ether, R_f 0.57 with 50%) to yield **92** as a clear oil (11 mg, 15 %).



4-(5-Hexen-1-yl)phenol (93). The procedure for the preparation of 4-(4-penten-1-yl)phenol (**92**) was adopted. Thus, **118** (0.20 g, 1.05 mmol) was treated with sodium thiomethoxide (0.74 g, 10.5 mmol) in DMF (20 mL) to give **93** as a viscous liquid (R_f 0.64 with 50% diethyl ether in petroleum ether, 93 mg, 50%). IR ($CHCl_3$ cast) 3346 (br m), 2928 (s), 1613 (m), 1513 (s), 1231 (s), 1172 (m), 910 (m), 827 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.05 (m, 2H, H-3) and 6.75 (m, 2H, H-2), 5.80 (m, 2H, H-5'), 5.00 (m, 2H, H-6'), 5.00-3.80 (br s, 1H, OH), 2.05 (t, 2H, J = 7.5 Hz, H-1'), 2.10 (m, 2H, H-4'), 1.60 (m, 2H, H-2'), 1.40 (m, 2H, H-3'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.43 (C-1), 138.92 (C-5'), 134.98 (C-4), 129.44 (C-3), 115.07 (C-2), 114.37 (C-6'), 34.87 (C-1'), 33.64 (C-4'), 31.15 (C-2'), 28.47 (C-3'); MS (EI) calcd for $C_{12}H_{16}O$ 176.1201, found 176.1198 (M^+, 31), 133.0652 (M-C_3H_5, 51), 107.0498 ($C_7H_7O^+$, 100), 91.0547 ($C_7H_8^+$, 9), 77.0391 ($C_6H_5^+$, 11); Anal. Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 81.53; H, 9.22.

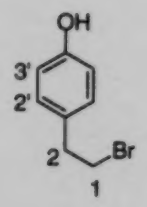


4-(6-Hepten-1-yl)phenol (94). The same procedure as for the preparation of 4-(4-penten-1-yl)phenol (**92**) was adopted. Thus, compound **119** (0.20 g, 0.98 mmol) was treated with sodium thiomethoxide (0.69 mg, 9.8 mmol) in DMF (20 mL). Purification by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.61 with 50%) afforded the product **94** as a yellow oil (0.1 g, 53%). IR ($CHCl_3$ cast) 3342 (br m), 2927 (s), 2855 (m), 1513 (s), 1232 (m), 1171 (m), 909 (m), 827 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.05 (m, 2H, H-3) and 6.75 (m, 2H, H-2), 5.80 (m, 1H, H-6'), 4.95 (m, 2H, H-7'), 5.1-3.9 (br s, 1H, OH), 2.55 (t, 2H, J = 7.6 Hz, H-1'), 2.05 (tq, 2H, J = 6.8, 1.3 Hz, H-5'), 1.60 (p, 2H, J = 7.6 Hz, H-2'), 1.35 (m, 4H, H-3', H-4'); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.41 (C-1), 139.09 (C-6'), 135.10 (C-4), 129.43 (C-3), 115.05 (C-2), 114.22 (C-7'), 34.97 (C-1'), 33.72 (C-5'), 31.55 (C-2'), 28.79 (C-4'), 28.72 (C-3'); MS (EI) calcd for $C_{13}H_{18}O$ 190.1358, found 190.1357 (M^+, 33), 133.0654 (M-C_4H_7, 40), 120.0574 (M-C_5H_9, 14), 107.0498 (M-C_6H_{11}, 100), 77.0392 ($C_6H_5^+$, 10); Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.53. Found: C, 82.04; H, 9.66.



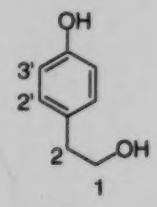
2-(4-Methoxyphenyl) ethanol (98). The procedure of Moreau and Rouessac was used.[145] To a suspension of lithium aluminum hydride (10 g, 263 mmol) in diethyl ether (150 mL) was slowly added a solution of 4-methoxyphenylacetic acid (33 g, 198 mmol) in diethyl ether (200 mL). The reaction mixture was heated under reflux overnight. Water (10 mL) was added, followed by 15% sodium hydroxide (10 mL) and water again (30 mL). The precipitate was filtered and the filtrate was washed with

water (2 × 300 mL), dried ($MgSO_4$) and evaporated *in vacuo*. The product **98** was obtained as an oil (26 g, 86%, R_f 0.21 with 50% diethyl ether in petroleum ether). IR ($CDCl_3$ cast) 3357 (br m), 2936 (m), 2911 (m), 1612 (m), 1513 (s), 1247 (s), 811 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, 2H, J = 8.2 Hz, H-2'), 6.80 (d, 2H, J = 8.2 Hz, H-3'), 3.85 (m, 5H, H-1, OCH_3), 2.80 (t, 2H, J = 6.4 Hz, H-2), 1.55 (br s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.30 (C-4'), 130.45 (C-1'), 129.97 (C-2'), 114.05 (C-3'), 63.83 (C-1), 55.28 (OCH_3), 38.28 (C-2); MS (EI) calcd for $C_9H_{12}O_2$ 152.0837, found 152.0840 (M^+, 26), 134.0728 (M-H_2O, 1), 121.0654 (M-OCH_3, 100), 107.0490 (M-C_2H_5O, 1), 77.0390 ($C_6H_5^+$, 9); Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 70.95; H, 8.10.



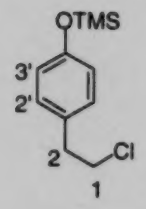
1-Bromo-2-(4-hydroxyphenl) ethane (99). Hydrogen bromide gas was bubbled through a solution of the compound **98** (5.0 g, 32 mmol) in methanol (300 mL) for 30 min. The solution was then heated under reflux for 2.5 days with hydrogen bromide still bubbling through it. Once the reaction mixture was cooled to r.t., water (100 mL) and diethyl ether (200 mL) were added. The aqueous layer was extracted with diethyl ether (3 × 400 mL). The combined organic layers were washed with water (2 × 750 mL) and with brine (750 mL), then dried ($MgSO_4$), concentrated *in vacuo*, and left under high vacuum for one day, affording **99** as a white solid (8.2 g, 90%, R_f 0.5 with 50% diethyl ether in petroleum ether). IR (acetone cast) 3359 (br m), 3062 (w), 3021 (w), 2963 (w), 1612 (m), 1514 (s), 1214 (s), 822 (m) cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 8.20 (s, 1H, OH), AA'XX' system 7.10 (m, 2H, H-2') and 6.70 (m, 2H, H-

3'), 3.65 (t, 2H, J = 7.5 Hz, H-1), 3.10 (t, 2H, J = 7.5 Hz, H-2); ^{13}C NMR (100 MHz, acetone-d_6) δ 156.78 (C-4'), 130.45 (C-1'), 130.24 (C-2'), 115.72 (C-3'), 38.89 (C-1), 34.20 (C-2); MS (EI) calcd for $C_8H_9{}^{81}BrO$ 201.9816, found 201.9822 (M^+, 21); for $C_8H_9{}^{79}BrO$ 199.9837, found 199.9843 (M^+, 22), 121.0650 (M-Br, 38), 107.0501 (M-CH_2Br, 100), 91.0547 ($C_7H_7^+$, 15), 77.0397 (16); Anal. Calcd for C_8H_9BrO: C, 47.79; H, 4.51. Found: C, 47.55; H, 4.40.

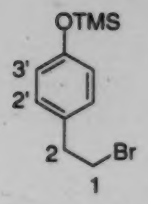


2-(4-hydroxyphenyl)ethanol (100). The procedure of Moreau and Rouessac was used.[145] A mixture of the protected phenol **98** (4.9 g, 32 mmol) and 30% hydrobromic acid in acetic acid (100 mL, 480 mmol) was heated under reflux overnight. Once cooled to r.t., the reaction mixture was diluted with diethyl ether (150 mL) and washed exhaustively with saturated sodium hydrogen carbonate (ca. 7 × 400 mL). The product was extracted with 5% sodium hydroxide (3 × 65 mL). The combined basic layers were acidified to pH 1 and extracted with diethyl ether (3 × 200 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The product **100** was purified by recrystallization from ethyl acetate/hexanes, affording beige crystals (0.67 g, 20%). IR (acetone cast) 3330 (br m), 2942 (m), 1612 (m), 1513 (s), 1241 (s), 823 (m) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.05 (s, 1H, ArOH), 7.0 (d, 2H, J = 8.4 Hz, H-2'), 6.7 (d, 2H, J = 8.4 Hz, H-3'), 3.7 (m, 3H, H-1, OH), 2.7 (t, 2H, J = 6.8 Hz, H-2); ^{13}C NMR (75 MHz, acetone-d_6) δ 156.49 (C-4'), 131.01 (C-1'), 130.66 (C-2'), 115.85 (C-3'), 64.22 (C-1), 39.44 (C-2); MS (EI) calcd for $C_8H_{10}O_2$ 138.0681,

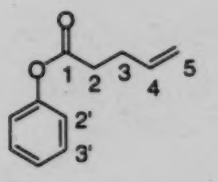
found 138.0678 ($M^{+\cdot}$, 26), 121.0648 (M-OH, 10), 107.0494 (M-CH_2OH, 100), 77.0391 ($C_6H_5^+$, 18).



2-(4-Trimethylsilyloxyphenyl)-1-chloroethane (101). The phenolic compound **99** (0.45 g, 2.2 mmol), trimethylsilyl chloride (0.5 mL, 3.6 mmol), and imidazole (0.25 g, 3.6 mmol) were dissolved in DMF (10 mL), and stirred at 40 °C for 1 day then at r.t. overnight. The reaction mixture was diluted in hexanes (50 mL), washed with water (3 × 40 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The product was purified by vacuum distillation, affording **101** as a colorless liquid (0.33 g, 66%). Bp 110 °C (12 mmHg); IR ($CHCl_3$ cast) 2958 (w), 1609 (w), 1511 (s), 1265 (s), 1252 (s), 915 (s), 847 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.80 (m, 2H, H-3'), 3.75 (t, 2H, J = 7.5 Hz, H-1), 3.00 (t, 2H, J = 7.5 Hz, H-2), 0.20 (s, 9H, $Si(C\underline{H}_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.10 (C-4'), 130.95 (C-1'), 129.77 (C-2'), 120.08 (C-3'), 45.15 (C-1), 38.51 (C-2); MS (EI) calcd for $C_{11}H_{17}{}^{37}ClOSi$ 230.0708, found 230.0705 ($M^{+\cdot}$, 2); for $C_{11}H_{17}{}^{35}ClOSi$ 228.0737, found 228.0732 ($M^{+\cdot}$, 5), 193.1041 (M-Cl, 0.5), 179.0891 (M-CH_2Cl, 14), 156.0319 (M-TMS, 24), 107.0493 ($C_7H_7O^+$, 100), 77.0398 ($C_6H_5^+$, 8); Anal. Calcd for $C_{11}H_{17}ClOSi$: C, 57.75; H, 7.49. Found: C, 57.84; H, 7.47.

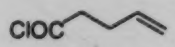


1-(4-Trimethylsilyloxyphenyl)-2-bromoethane (102). Compound **99** (1 g, 5 mmol), trimethylsilyl chloride (0.95 mL, 7.5 mmol), and pyridine (0.74 mL, 7.5 mmol) were dissolved in THF (10 mL) and stirred at r.t. overnight. The reaction mixture was filtered and the filtrate was evaporated. The product was purified by vacuum distillation to yield a colorless liquid **102** (0.54 g, 64%). Bp 98 °C (< 1 mm Hg); IR ($CHCl_3$ cast) 2960 (m), 1610 (m), 1511 (s), 1265 (s), 1252 (s), 916 (s), 847 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ AA'XX' system 7.05 (m, 2H, H-3') and 6.80 (m, 2H, H-2'), 3.50 (t, 2H, J = 7.8 Hz, H-2), 3.10 (t, 2H, J = 7.8 Hz, H-1), 0.20 (s, 9H, $Si(C\underline{H}_3)_3$; MS (EI) calcd for $C_{11}H_{17}{}^{81}BrOSi$ 274.02115, found 274.02213 (M^+, 30); for $C_{11}H_{17}{}^{79}BrOSi$ 272.0232, found 272.0239 (M^+, 30), 179.0898 (M-CH_2Br, 100), 121.0655 ($C_8H_9O^+$, 33), 73.0476 (Me_3Si^+, 59).

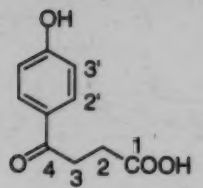


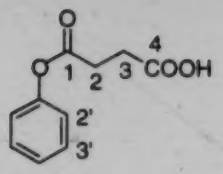
Phenyl pent-4-enoate (103). The known[181] compound **103** was prepared as follows. 4-Pentenoyl chloride (**104**) (0.7 mL, 6.4 mmol) and phenol (1.6 g, 17.5 mmol) were mixed and stirred at r.t for two hours. The reaction mixture was diluted with chloroform (ca. 20 mL) and extracted with 10% sodium hydroxide (4 × 25 mL) until the organic layer was colorless. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by silica gel flash column

chromatography (0 to 10% diethyl ether in petroleum ether, R_f 0.45 with 10%) to give compound **103** as a colorless oil (1.1 g, 98%). IR ($CHCl_3$ cast) 3078 (w), 3043 (w), 2980 (w), 2921 (w), 1760 (s), 1641 (m), 1363 (m), 1197 (s), 752 (m), 713 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.37 (tt, 2H, J = 7.5, 1.5 Hz, H-3'), 7.23 (tt, 1H, J = 7.5, 1.5 Hz, H-4'), 7.07 (br d, 2H, J = 7.5 Hz, H-2'), 5.91 (ddt, 1H, J = 13.5, 10.2, 7.2 Hz, H-4), 5.11 (m, 2H, H-5), 2.68 (dt, 2H, J = 7.2, 1.0 Hz, H-2), 2.52 (ddq, 2H, J = 7.2, 1.0, 1.0 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.53 (C-1), 150.73 (C-1'), 136.34 (C-4), 129.40 (C-3'), 125.78 (C-4'), 121.57 (C-2'), 115.93 (C-5), 33.66 (C-2), 28.90 (C-3); MS (EI) calcd for $C_{11}H_{12}O_2$ 176.0837, found 176.0840 (M^+, 19), 94.0400 ($C_6H_6O^+$, 100); Anal. Calcd for $C_{11}H_{12}O_2$: C, 74.98; H, 6.87. Found: C, 74.81; H, 6.85.



4-Pentenoyl chloride (104). 4-Pentenoic acid (10 mL, 98 mmol) was added dropwise to thionyl chloride (8.2 mL, 112 mmol). The mixture was then heated at 100 °C for one hour. The product was purified by distillation, affording 4-pentenoyl chloride (**104**) as a colorless liquid (9.6 g, 83%). Bp 123 °C; IR ($CHCl_3$ cast) 3440 (br m), 3083 (m), 2983 (m), 2923 (m), 1798 (s), 1642 (m), 996 (s), 958 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (ddt, 1H, J = 13.5, 10.2, 6.5 Hz, H-4), 5.10 (m, 2H, H-5), 3.05 (t, 2H, J = 7.2 Hz, H-2), 2.50 (dd, 2H, J = 7.2, 6.5 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.13 (C-1), 134.65 (C-4), 116.88 (C-5), 46.21 (C-2), 28.88 (C-3); GC-MS (10% $CHCl_3$ solution) 120.1 ($^{37}ClM^+$, 0.3), 118.1 (M^+, 1), 92.1 (^{37}ClM-CO, 8) 90.1 (M-CO, 21), 83.2 (M-Cl, 87), 55.1 (M-COCl, 100), 39.0 ($C_3H_3^+$, 65).



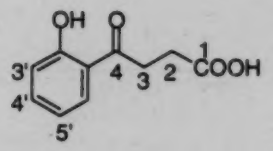


4-(4-Hydroxyphenyl)-4-oxobutyric acid (105) and phenyl 3-carboxypropanoate (107). To a solution of aluminum chloride (15 g, 112 mmol) in nitrobenzene (40 mL) at 0 °C was added a solution of phenol (3.3 g, 35 mmol) in nitrobenzene (7 mL) followed by succinic anhydride (4.1 g, 41 mmol). The mixture was stirred at r.t. overnight (ca. 50% completion). The reaction mixture was poured into concentrated HCl and ice, and the organic layer was extracted with saturated sodium hydrogen carbonate (2 × 200 mL). Most of the nitrobenzene present in the aqueous layer was washed off with diethyl ether. After acidification to pH 1 (2N HCl), the aqueous layer was extracted with diethyl ether (4 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. Purification by silica gel column chromatography (30% petroleum ether in diethyl ether) afforded **107** as a white solid (R_f 0.33, 0.6 g, 15%), compound **105** as a white solid (R_f 0.09, 0.2 g, 10%).

Data for 4-(4-hydroxyphen-1-yl)-4-oxobutyric acid (105).[182] IR (CH_2Cl_2 cast) 3281 (br m), 2917 (m), 2848 (m), 1707 (s), 1654 (s), 1511 (s), 1217 (s) cm^{-1}; ^1H NMR (360 MHz, acetone-d_6) δ 9.80 (s, 1H, OH), AA'XX' system 7.90 (m, 2H, H-2') and 6.90 (m, 2H, H-3'), 3.2 (t, 2H, J = 6.5 Hz, H-3), 2.70 (t, 2H, J = 6.5 Hz, H-2); ^{13}C NMR (75 MHz, acetone d_6) δ 196.83 (C-4), 174.22 (C-1), 162.63 (C-4'), 131.19 (C-2'), 130.05 (C-1'), 115.98 (C-3'), 33.42 (C-3), 28.30 (C-2); MS (EI) calcd for $C_{10}H_{10}O_4$ 194.0579, found 194.0575 (M^+, 11), 177.0553 (M-OH, 2), 121.0288 ($C_7H_5O_2^+$, 100), 93.0343 ($C_6H_5O^+$, 15); Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 62.16; H, 5.25.

Data for phenyl 3-carboxypropanoate (107).[183] IR ($CHCl_3$ cast) 3055 (m), 2941 (m), 1758 (s), 1700 (s), 1320 (s), 1146 (s), 768 (m), 689 (m) cm^{-1}; ^1H NMR (300

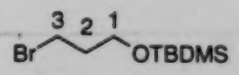
MHz, $CDCl_3$) δ 7.35 (m, 2H, H-3'), 7.25 (tt, 2H, J = 8.4, 1.1 Hz, H-4'), 7.10 (m, 2H, H-2'), 2.90 (m, 2H, H-2), 2.80 (m, 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.92 (C-4), 170.72 (C-1), 150.58 (C-1'), 129.44 (C-3'), 125.93 (C-4'), 121.45 (C-2'), 29.06, 28.74 (C-2, C-3); MS (EI) calcd for $C_{10}H_{10}O_4$ 194.0579, found 194.0591 (M^+, 15), 94.0421 ($C_6H_5O^+$, 100); Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.30; H, 5.13.



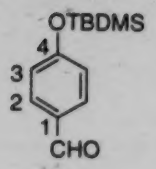
4-(2-Hydroxyphen-1-yl)-4-oxobutyric acid (106). The known[184] compound **106** was prepared as follows. To a solution of aluminum chloride (18 g, 135 mmol) in nitrobenzene (40 mL) cooled to 0 °C was added dropwise a solution of phenol (3.1 g, 33 mmol) in nitrobenzene (10 mL), followed by succinic anhydride (3.5 g, 35 mmol). The mixture was stirred at r.t. for one day, then heated at 100 °C for three hours. The cooled mixture was poured into concentrated HCl and ice. The organic layer was extracted with 10% NaOH (3 × 70 mL). The combined basic layers were acidified to pH 2 (1N HCl) and extracted with diethyl ether (4 × 50 mL). The combined diethyl ether layers were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by silica gel flash chromatography (30% petroleum ether in diethyl ether, R_f 0.16), to yield compound **106** as a pinkish white solid (0.2 g from half of the mixture, 10%). IR ($CHCl_3$ cast) 3028 (br w), 2921 (w), 1703 (s), 1642 (s), 1157 (s), 758 (s) cm^{-1}, ^1H NMR (360 MHz, $CDCl_3$) δ 12.05 (s, 1H, $CO_2\underline{H}$), 7.80 (dd, 1H, J = 8.2, 1.5 Hz, H-6'), 7.50 (ddd, 1H, J = 8.5, 7.2, 1.5 Hz, H-4'), 7.00 (dd, 1H, J = 8.5, 1.1 Hz, H-3'), 6.90 (ddd, 1H, J = 8.2, 7.2, 1.1 Hz, H-5'), 3.40 (t, 2H, J = 6.5 Hz, H-3), 2.80 (t, 2H, J = 6.5 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.54 (C-4), 177.63 (C-1), 167.29 (C-2'), 136.56 (C-1'), 129.67 (C-4'), 119.06 (C-5', C-6'), 118.59 (C-3'), 32.84 (C-3), 27.49 (C-2); MS (EI)

calcd for $C_{10}H_{10}O_4$ 194.0579, found 194.0584 (M^+, 24), 176.0475 (M-H_2O, 21), 148.0521 (M-CH_2OH, 14), 121.0290 ($C_7H_5O_2^+$, 100), 93.0340 ($C_6H_5O^+$, 19); Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.47; H, 4.86.

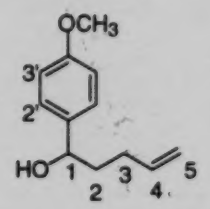
Alternative procedure for the preparation of 4-(2-hydroxyphen-1-yl)-4-oxobutyric acid (106). The method used for the preparation of compounds **105** and **107** also affords compound **106** (R_f 0.16, 10 mg, 0.05 %) with identical spectroscopic data as above.



3-Bromo-1-*tert*-butyldimethylsilyloxypropane (108). To a solution of 3-bromopropanol (3.0 mL, 33.2 mmol) in THF (30 mL) was added pyridine (4 mL, 50 mmol), followed by *tert*-butyldimethylsilyl chloride (7.5 g, 50 mmol). The mixture was stirred at r.t. overnight, poured into a mixture of concentrated HCl and ice (ca. 60 mL), and extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.78 with 50%) to yield **108** (3.6 g, 50%). IR ($CHCl_3$ cast) 2955 (s), 2929 (s), 1557 (s), 1103 (s), 836 (s), 777 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 (t, 2H, J = 5.7 Hz, H-1), 3.05 (t, 2H, J = 6.5 Hz, H-3), 2.05 (m, 2H, H-2), 0.80 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.05 (s, 6H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 60.43 (C-1), 35.56 (C-3), 30.66 (C-2), 25.90 ($SiC(\underline{C}H_3)_3$, 18.30 ($Si\underline{C}(CH_3)_3$), -5.38 ($Si(\underline{C}H_3)_2$; MS (EI) calcd for $C_5H_{12}{}^{81}BrOSi$ 196.9820, found 196.9823 (M-C_4H_9, 100); calcd for $C_5H_{12}{}^{79}BrOSi$ 194.9841, found 194.9844 (M-C_4H_9, 98).

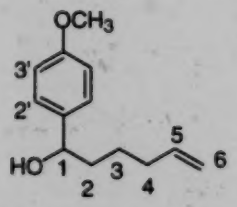


4-*tert*-Butyldimethylsilyloxybenzaldehyde (109). To a solution of 4-hydroxybenzaldehyde (2.0 g, 16.4 mmol) and imidazole (1.5 g, 22 mmol) in DMF (10 mL) was added *tert*-butyldimethylsilyl chloride (3.7 g, 25 mmol). After stirring for two days at r.t., the mixture was diluted with hexanes (ca. 130 mL) and washed with water (3 × 100 mL). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. Purification of the residue by silica gel chromatography (20% diethyl ether in petroleum ether R_f 0.64 with 50%) afforded **109** (3.3 g, 87%) as a clear oil. IR ($CHCl_3$ cast) 2930 (m), 1700 (s), 1599 (s), 1508 (s), 1273 (s), 909 (s), 841 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.90 (s, 1H, CHO), AA'XX' system 7.80 (m, 2H, H-2) and 6.90 (m, 2H, H-3), 1.00 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.20 (s, 6H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.82 (CHO), 161.50 (C-4), 131.88 (C-4), 10.46 (C-1), 120.47 (C3), 25.55 ($SiC(\underline{C}H_3)_3$), 18.24 ($Si\underline{C}(CH_3)_3$), -4.37 ($Si(\underline{C}H_3)_2$); MS (EI) calcd for $C_{13}H_{20}O_2Si$ 236.1232, found 236.1233 (M^+, 36), 179.0528 (M-C_4H_9, 100); Anal. Calcd for $C_{13}H_{20}O_2Si$: C, 66.05; 8.53. Found: C, 65.64; H, 8.64.



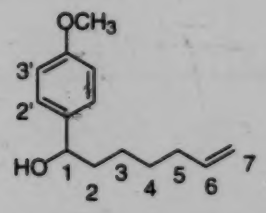
1-(4-Methoxyphenyl)-4-penten-1-ol (114). The known[185] compound **114** was prepared as follows. Magnesium turnings (0.4 g, 16.7 mmol) were activated by drying overnight in the oven and vigorously stirring under Ar for 20 min. To a suspension of

activated magnesium turnings in THF (5 mL) was added 4-bromo-1-butene (**111**) (2.0 g, 14.8 mmol). The reaction was highly exothermic. The mixture was stirred without heating for 1.5 hour. *p*-Anisaldehyde (2.0 g, 14.8 mmol) was added slowly. The reaction mixture was stirred without heat for 30 min, heated under reflux for 3 hours, then stirred at r.t. overnight. To the orange solution obtained was added water and 1N HCl (5 mL each). The mixture was extracted with diethyl ether (3 × 20 mL) and the combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The product **114** was purified by vacuum distillation, affording a colorless oil (1.64 g, 61%, R_f 0.45 with 50% diethyl ether in petroleum ether). Bp 112 °C (<1 mm Hg); IR ($CHCl_3$ cast) 3450 (br m), 3075 (w), 2925 (m), 1611 (m), 1512 (s), 1247 (s), 1172 (s), 1034 (s), 1004 (s), 915 (s), 831 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.30 (m, 2H, H-2') and 6.90 (m, 2H, H-3'), 5.90 (m, 1H, H-4), 5.05 (m, 2H, H-5), 4.60 (br t, 1H, J = 7.5 Hz, H-1), 3.80 (s, 3H, OCH_3), 2.20-1.50 (m, 5H, H-2, H-3 and OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.22 (C-4'), 136.77 (C-1'), 127.14 (C-2'), 114.87 (C-5), 113.88 (C-3'), 113.72 (C-4), 73.67 (C-1), 55.28 (OCH_3), 37.98 (C-3), 30.12 (C-2); MS (EI) calcd for $C_{12}H_{16}O_2$ 192.1150, found 192.1147 (M^+, 6), 175.1115 (M-OH, 13), 165.0915 (M-$C_2H_3^+$, 44), 137.0602 (M-$C_4H_7^+$, 100), 121.0653 (M-$C_4H_7O^+$, 41), 94.0417 ($C_6H_6O^+$, 11), 77.0390 ($C_6H_5^+$, 29); Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.84; H, 8.23.



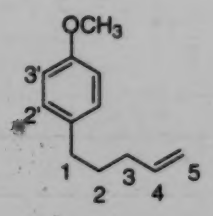
1-(4-Methoxyphenyl)-5-hexen-1-ol (115). The known[186] compound **115** was prepared by the method used for the preparation of 1-(4-methoxyphenyl)-4-penten-1-ol

(**114**) was adopted. Thus, 5-bromo-1-pentene (**112**) (1.6 mL, 13.5 mmol) was treated with magnesium turnings (0.49 g, 20.3 mmol) in THF before the addition of *p*-anisaldehyde (1.6 mL, 13.2 mmol). Purification by distillation afforded the elimination product (0.65 g, 26%) as well as the desired product **115** (1.7 g, 62%). Purification by silica gel column chromatography (0 to 25% diethyl ether in petroleum ether, R_f 0.42 with 50%) afforded the product **115** as a colorless oil (1.6 g, 60%). IR ($CDCl_3$ cast) 3450 (br w), 2934 (m), 1611 (m), 1513 (s), 1246 (s), 1174 (m), 1036 (s), 831 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.30 (m, 2H, H-2') and 6.90 (m, 2H, H-3'), 5.80 (m, 1H, H-5), 4.95 (m, 2H, H-6), 4.65 (t, 1H, J = 6.4 Hz, H-1), 3.85 (s, 3H, OCH_3), 2.10 (m, 2H, H-2), 1.90-1.60 (m, 3H, H-4, OH), 1.60-1.15 (m, 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.06 (C-4'), 138.60 (C-5), 137.00 (C-1'), 127.12 (C-2'), 114.64 (C-6), 113.84 (C-3'), 74.15 (C-1), 55.28 (OCH_3), 38.38 (C-4), 33.58 (C-2), 25.15 (C-3); MS (EI) calcd for $C_{13}H_{18}O_2$ 206.1307, found 206.1302 (M^+, 17), 189.1275 (M-OH, 3), 163.0759 (M-C_3H_7, 16), 137.0602 (M-C_5H_9, 100), 109.0654 ($C_7H_9O^+$, 33), 94.0420 ($C_6H_6O^+$, 18), 77.0394 ($C_6H_5^+$, 23); Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.80. Found: C, 75.53; H, 8.77.



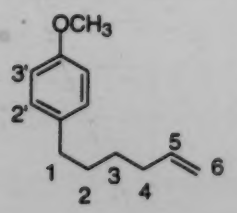
1-(4-Methoxyphenyl)-6-hepten-1-ol (116). The known[187] compound **116** was prepared using the same method as for 1-(4-methoxyphenyl)-4-penten-1-ol (**114**). 6-Bromo-1-hexene (**113**) (0.7 mL, 5.2 mmol) was treated with magnesium turnings (0.44 g, 18.5 mmol) in THF followed by the addition of *p*-anisaldehyde (1.65 mL, 5.3 mmol). Purification by silica gel column chromatography (0 to 25% diethyl ether in petroleum

ether, R_f 0.29 with 66%) afforded the product **116** as a slightly yellow oil (1.1 g, 97%). IR (CH_2Cl_2 cast) 3440 (br w), 2932 (s), 1611 (m), 1512 (s), 1247 (s), 1173 (m), 1037 (s), 832 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.25 (m, 2H, H-2') and 6.90 (m, 2H, H-3'), 5.80 (m, 1H, H-6), 4.95 (m, 2H, H-7), 4.65 (t, 1H, J = 6.6 Hz, H-1), 3.80 (s, 3H, $OC\underline{H}_3$), 2.05 (tq, 2H, J = 6.6, 1.3 Hz, H-2), 1.90-1.05 (m, 3H, H-5, OH), 1.45 (m, 2H, H-3), 1.25 (m, 2H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.06 (C-4'), 138.87 (C-6), 137.03 (C-1'), 127.13 (C-2'), 114.36 (C-7), 113.84 (C-3'), 74.24 (C-1), 55.29 ($O\underline{C}H_3$), 38.81 (C-2), 33.67 (C-5), 28.81 (C-3), 25.40 (C-4); MS (EI) calcd for $C_{14}H_{20}O_2$ 220.1463, found 220.1462 (M^+, 10), 202.1355 (M-H_2O, 1), 190.1354 (M-CH_2O, 12), 165.0915 (M-C_4H_7, 7), 137.0602 (M-C_6H_{11}, 100), 121.0653 ($C_8H_9O^+$, 36), 94.0419 ($C_6H_6O^+$, 4), 77.0391 ($C_6H_5^+$, 8); Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15. Found: C, 76.43; H, 9.16.



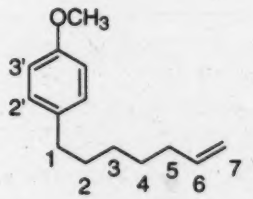
1-(4-Methoxyphenyl)-4-pentene (117). A modification of the procedure of Harris *et al.* was used.[151] To a suspension of lithium aluminum hydride (0.2 g, 5 mmol) in diethyl ether (10 mL) was slowly added a solution of aluminum chloride (0.69 g, 5 mmol) in diethyl ether (4 mL). The reaction was exothermic. The mixture was stirred at r.t. for 25 min before the addition of a solution of compound **114** (0.1 g, 0.5 mmol) in diethyl ether (4.5 mL). The mixture was stirred at r.t. overnight, then cooled to 0 °C, at which time ethyl acetate (9 mL), water (6 mL), and conc. hydrochloric acid (6 mL) were added dropwise. The resulting heterogeneous mixture was stirrred a r.t. for one hour, then the aqueous layer was extracted with diethyl ether (5 × 25 mL). The

combined organic layers were washed with a solution of saturated sodium hydrogen carbonate (6 × 100 mL) and with brine (100 mL), then dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (25% diethyl ether in petroleum ether, R_f 0.81 with 50%) to give the product **117** as a colorless oil (88 mg, 98%). IR ($CHCl_3$ cast) 2931 (m), 1612 (m), 1512 (s), 1246 (s), 1176 (m), 1038 (m), 911 (m), 831 (m), 808 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.80 (m, 2H, H-3'), 5.80 (m, 1H, H-4), 5.00 (m, 2H, H-5), 3.85 (s, 3H, OCH_3), 2.60 (t, 2H, J = 7.1 Hz, H-1), 2.15 (br q, 2H, J = 7.1 Hz, H-3), 1.75 (br p, 2H, J = 7.1 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.72 (C-4'), 138.68 (C-4), 134.56 (C-1'), 129.30 (C-2'), 114.61 (C-5), 113.72 (C-3'), 55.25 (OCH_3), 34.38 (C-1), 33.22 (C-3), 30.84 (C-2); MS (EI) calcd for $C_{12}H_{16}O$ 176.1201, found 176.1200 (M^+, 37), 147.0811 (M-Et, 7), 134.0732 (M-C_3H_5, 78), 121.0653 (M-C_4H_7, 100), 91.0547 ($C_7H_9O^+$, 12), 77.0391 ($C_6H_5^+$, 10); Anal. Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found C, 81.28; H, 9.23.

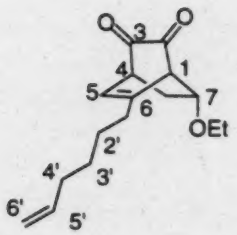


1-(4-Methoxyphenyl)-5-hexene (118). Prepared using the same method as for 1-(4-methoxyphenyl)-4-pentene (**117**). The alcohol **115** (0.5 g, 2.4 mmol) was treated with aluminum chloride (3.2 g, 24 mmol) and lithium aluminum hydride (0.92 g, 24 mmol) in diethyl ether and the product **118** was obtained as a yellow oil (R_f 0.78 with 50% diethyl ether in petroleum ether, 0.4 g, 87%). IR ($CHCl_3$ cast) 2930 (m), 2855 (w), 1612 (w), 1512 (s), 1464 (w), 1245 (s), 1176 (m), 910 (w), 827 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.15 (m, 2H, H-2') and 6.85 (m, 2H, H-3'), 5.85 (m, 1H,

H-5), 5.00 (m, 2H, H-6), 3.80 (s, 3H, OC<u>H</u>$_3$), 2.55 (t, 2H, J = 7.5 Hz, H-1), 2.05 (q, 2H, J = 7.1 Hz, H-4), 1.60 (m, 2H, H-2), 1.40 (p, 2H, J = 7.1 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.67 (C-4'), 138.93 (C-5), 134.80 (C-1'), 129.25 (C-2'), 114.35 (C-6), 113.69 (C-3'), 55.25 (O<u>C</u>H$_3$), 34.87 (C-1), 33.65 (C-4), 31.18 (C-2), 28.50 (C-3); MS (EI) calcd for $C_{13}H_{18}O$ 190.1358, found 190.1359 (M^+, 54), 161.0965 (M-Et, 2), 147.0811 (M-Pr, 53), 121.0654 (M-C_5H_9, 100), 91.0547 ($C_7H_8^+$, 7), 77.0392 ($C_6H_5^+$, 7); Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.53. Found: C, 81.47; H, 9.70.

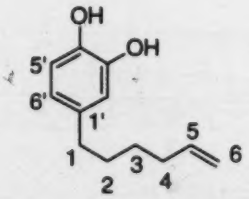


1-(4-Methoxyphenyl)-6-heptene (119). The method used for the preparation of 1-(4-methoxyphenyl)-4-pentene (**117**) was adopted. Thus, **116** (0.50 g, 2.27 mmol) was treated with aluminum chloride (3.0 g, 22.7 mmol) and lithium aluminum hydride (0.86 g, 22.7 mmol) in diethyl ether to yield the product **119** as a yellow oil (R_f 0.78 with 50% diethyl ether in petroleum ether, 0.31 g, 67%). IR ($CHCl_3$ cast) 2928 (m), 2854 (m), 1512 (s), 1246 (s), 1176 (m), 1039 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.85 (m, 2H, H-3'), 5.80 (m, 1H, H-6), 5.00 (m, 2H, H-7), 3.80 (s, 3H, OC<u>H</u>$_3$), 2.55 (t, 2H, J = 7.5 Hz, H-1), 2.05 (tq, 2H, J = 10.5, 1.6 Hz, H-5), 1.60 (p, 2H, J = 7.5 Hz, H-2), 1.50-1.20 (m, 4H, H-3, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.64 (C-4'), 139.10 (C-6), 134.93 (C-1'), 129.23 (C-2'), 114.21 (C-7), 113.67 (C-3'), 55.25 (O<u>C</u>H$_3$), 34.97 (C-1), 33.73 (C-5), 31.58 (C-2), 28.81, 28.75 (C-3, C-4); MS (EI) calcd for $C_{14}H_{20}O$ 204.1514, found 204.1515 (M^+, 42), 161.0966 (M-C_3H_7, 2), 147.0813 (M-C_4H_9, 42), 121.0655 ($C_8H_9O^+$, 100), 91.0547 ($C_7H_8^+$, 9), 77.0391 ($C_6H_5^+$, 9); Anal. Calcd for $C_{14}H_{20}O$: C, 82.30; H, 9.87. Found: C, 81.50; H, 9.81.



***exo*-7-Ethoxy-6-(5-hexen-1-yl)bicyclo[2.2.2]-2,3-dioxo-octa-5-ene (120).** Mushroom tyrosinase (EC 1.14.18.1, activity of 3400 units/mg solid) was immobilized on glass beads as described for 2-(4-penten-1-yl)phenol (**92**) (see enzymatic tests section). The 106 microns and finer glass beads (10.9 g) were washed with buffer (0.05 M phosphate buffer, pH 7.0) and dried under high vacuum. A solution of enzyme (82 mg, 129 kU) in buffer (4 mL) was poured over the dry glass beads and dried. A blank reaction mixture was prepared by mixing 4-(5-hexen-1-yl)phenol (**93**) (6 mg, 0.034 mmol) with dry chloroform (3 mL) and buffer (0.005 mL). The enzymatic reaction was started by adding immobilized tyrosinase (5 g, 59 kU) to a solution of substrate **93** (20 mg, 0.11 mmol) in dry chloroform (20 mL) and buffer (0.03 mL). The reaction mixtures were left on an orbital shaker at r.t. and 150 rpm for two days. An additional amount of immobilized enzyme (9.5 g, 245 kU) and buffer (0.01 mL) were added and the mixture was left on the shaker at r.t. and 100 rpm for 3 more days (time required to react all compound **93**). Ethyl vinyl ether was then added to both the blank (5 mL) and the enzymatic reaction (10 mL), and the mixtures were allowed to shake at 100 rpm and r.t. for one day. Evaporation of the solvent afforded pure recovered material **93** for the blank (5.0 mg, 85%). For the enzymatic reaction, the enzyme was filtered off and washed with chloroform. The filtrate was dried ($MgSO_4$), and the residue obtained was further purified by silica gel flash column chromatography (20% diethyl ether in petroleum ether, R_f 0.59 with 50%). The product **120** was obtained as a yellow oil (7.9

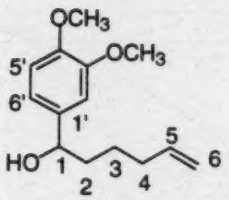
mg, 30%). After 3 days at -4 °C the compound was about 30% degraded. $[\alpha]_D^{25} = 0.000$ (*c* 0.2, $CHCl_3$); IR (CH_2Cl_2 cast) 2973 (s), 2930 (s), 1732 (s), 1713 (s), 1451 (s), 1198 (s), 1161 (s), 1115 (s), 1099 (s), 1026 (s), 929 (s), 910 (s) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.64 (d, 1H, *J* = 8.2 Hz, H-4), 6.54 (d, 1H, *J* = 8.2 Hz, H-5), 5.76 (m, 2H, H-5', H-7), 4.95 (tdd, 1H, *J* = 17.0, 2.0, 1.7 Hz, H_a-6'), 4.89 (br dd, 1H, *J* = 10.2, 2.0 Hz, H_b-6'), 4.60 (br s, 1H, H-1), 3.89 (dq, 1H, *J* = 9.6, 7.1 Hz, OCH$\underline{H}^a$), 3.61 (qd, 1H, *J* = 9.6, 7.1 Hz, OCH$\underline{H}^b$), 3.25 (dd, 1H, *J* = 16.4, 6.9 Hz, H-8 up), 2.96 (dd, 1H, *J* = 16.4, 2.4 Hz, H-8 down), 2.42 (t, 2H, *J* = 7.8 Hz, H-1'), 2.03 (br q, 2H, *J* = 7.2 Hz, H-4'), 1.51 (m, 2H, H-2'), 1.38 (br p, 2H, *J* = 7.2 Hz, H-3'), 1.20 (t, 3H, *J* = 7.1 Hz, C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.023 (C-2), 139.43 (C-5'), 138.72 (C-3), 131.63 (C-6), 125.22 (C-1), 121.93 (C-5), 115.31 (C-4), 114.84 (C-6'), 107.46 (C-7), 64.83 (O$\underline{C}H_2$), 36.93 (C-8), 34.15 (C-4'), 33.16 (C-1'), 30.23 (C-2'), 29.19 (C-3'), 15.59 ($\underline{C}H_3$); MS (EI) calcd for $C_{16}H_{22}O_3$ 262.1569, found 262.1569 (M^+, 100), 217.1226 (M-OEt, 5), 193.0870 (M-C_5H_9, 17), 175.0757 (M-$C_5H_{11}O$, 42), 165.0553 ($C_9H_9O_3^+$, 51), 147.0445 ($C_9H_7O_2^+$, 45), 137.0602 ($C_8H_9O_2^+$, 33).



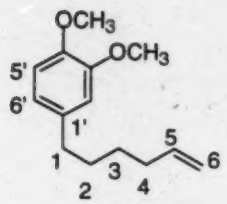
1-(3,4-Dihydroxyphenyl)-5-hexene (121). Compound **123** (0.49 g, 2.2 mmol) and sodium thiomethoxide (3.2 g, 45 mmol) were dissolved in DMF (60 mL) at r.t. The mixture was then stirred at 120-150 °C for 5.5 days. Once the mixture was cooled to r.t., it was acidified to pH 1 (2N HCl) and heated to evaporate the methanethiol formed. The solution was extracted with diethyl ether (3 × 60 mL) and the combined organic layers were washed with water (150 mL), with 0.01M silver nitrate (150 mL), with

water again (150 mL), and with brine (150 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. Purification by silica gel column chromatography (33 to 50% diethyl ether in petroleum ether, R_f 0.42 with 50%) afforded the product **121** as a clear oil (0.16 g, 38%). IR ($CHCl_3$ cast) 3362 (br s), 2855 (m), 1605 (m), 1519 (s), 1443 (m), 1282 (s), 1260 (m), 1191 (s), 1112 (m), 910 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.75 (d, 1H, J = 8.0 Hz, H-5'), 6.70 (d, 1H, J = 2.0 Hz, H-2'), 6.60 (dd, 1H, J = 8.0, 2.0 Hz, H-6'), 5.80 (m, 1H, H-5), 5.00 (m, 2H, H-6), 2.50 (t, 2H, J = 7.5 Hz, H-1), 2.10 (tq, 2H, J = 7.2, 1.2 Hz, H-4), 1.60 (m, 2H, H-2), 1.40 (m , 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.34 (C-3'), 141.22 (C-4'), 138.87 (C-5), 136.08 (C-1'), 120.83 (C-6'), 115.52 (C-2'), 115.26 (C-5'), 114.37 (C-6), 35.05 (C-1), 33.61 (C-4), 30.98 (C-2), 28.43 (C-3); MS (EI) calcd for $C_{12}H_{16}O_2$ 192.1150, found 192.1147 (M^+, 51), 149.0605 (M-C_3H_7, 23), 136.0525 (M-C_4H_7, 22), 123.0445 (M-C_5H_9, 100), 110.0365 (M-C_6H_{10}, 7), 91.0550 ($C_7H_7^+$, 4), 77.0390 ($C_6H_5^+$, 13); Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.62; H, 8.50.

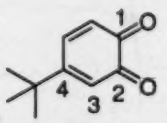
Oxidation of 1-(3,4-dihydroxyphenyl)-5-hexene (121) with silver carbonate on Celite (124). Silver carbonate on Celite (**124**) (50 g, ca. 1 mmol), prepared as described later, was azeotropically dried by distillation of benzene (200 mL). The greenish yellow solid was then resuspended in dichloromethane (150 mL) and a solution of the catechol **121** (0.05 g, 0.26 mmol) in dichloromethane (1.5 mL) was added. The mixture turned from yellow to brown. It was stirred for five hours at r.t., after which, TLC showed no more starting material **121** (R_f 0.06 with 100% dichloromethane) but a single new spot (R_f 0.16). The brown solid was vacuum filtered, and the filtrate was evaporated *in vacuo*. According to NMR and TLC, the product (R_f 0.16) had already polymerized.



1-(3,4-Dimethoxyphenyl)-5-hexen-1-ol (122). The procedure for the preparation of 1-(4-methoxyphenyl)-4-penten-1-ol (**114**), was adopted. Thus, 5-bromo-1-pentene (**112**) (1.6 mL, 13.5 mmol) was treated with magnesium turnings (0.49 g, 20.3 mmol) in THF before the addition of 3,4-dimethoxybenzaldehyde (2.24 g, 13.5 mmol). Purification by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.21 with 50%) afforded the product **122** as a colorless oil (2.5 g, 78%). IR ($CHCl_3$ cast) 3400 (br w), 2935 (m), 1516 (s), 1261 (s), 1233 (s), 1028 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 (d, 1H, J = 1.6 Hz, H-2'), 6.85 (m, 2H, H-5', H-6'), 5.75 (m, 1H, H-5), 5.00 (m, 2H, H-6), 4.60 (dd, 1H, J = 7.4, 6.1 Hz, H-1), 3.85 (s, 3H, $OC\underline{H}_3$), 3.88 (s, 3H, $OC\underline{H}_3$), 2.10 (tdd, 1H, J = 14.2, 7.4, 1.3 Hz, H_a-2), 2.08 (tdd, 1H, J = 14.2, 6.1, 1.3 Hz, H_b-2), 1.80-1.25 (m, 5H, OH, H-3, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.10 (C-3'), 148.44 (C-4'), 138.58 (C-5), 137.52 (C-1'), 118.15 (C-2'), 114.68 (C-6), 110.94 (C-6'), 108.96 (C-5'), 74.40 (C-1), 55.93 ($O\underline{C}H_3$), 55.87 ($O\underline{C}H_3$), 38.41 (C-4), 33.56 (C-2), 25.18 (C-3); MS (EI) calcd for $C_{14}H_{20}O_3$ 236.1413, found 236.1412 (M^+, 71), 218.1306 (M-H_2O, 15), 193.0863 (M-C_3H_7, 30), 177.0914 (M-C_3H_7O, 26), 167.0719 (M-C_5H_9, 100), 139.0764 (M-C_6H_9O, 83), 124.0525 ($C_7H_8O_2^+$, 25), 108.0576 ($C_7H_8O^+$, 19), 77.0394 ($C_6H_5^+$, 15); Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 71.30; H, 8.75.

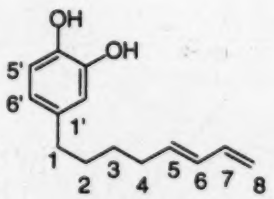


1-(3,4-Dimethoxyphenyl)-5-hexene (123). The procedure used for the preparation of 1-(4-methoxyphenyl)-4-pentene (**117**) was adopted. Compound **122** (1.0 g, 4.2 mmol) was treated with aluminum chloride (3.2 g, 24 mmol) and lithium aluminum hydride (0.92 g, 24 mmol) in diethyl ether to afford the product **123** as a yellow oil (R_f 0.53 in 50% diethyl ether in petroleum ether, 0.83 g, 89%). IR ($CHCl_3$ cast) 2931 (s), 1516 (s), 1262 (s), 1237 (s), 1031 (s), 803 (m), 849 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (d, 1H, J = 8.6 Hz, H-5'), 6.70 (m, 2H, H-2', H-6'), 5.80 (m, 1H, H-5), 4.95 (m, 2H, H-6), 3.90 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.55 (t, 2H, J = 7.5 Hz, H-1), 2.10 (tq, 2H, J = 7.1, 1.3 Hz, H-4), 1.65 (m, 2H, H-2), 1.45 (m, 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.78 (C-3'), 147.06 (C-4'), 138.90 (C-5), 135.39 (C-1'), 120.14 (C-2'), 114.39 (C-6), 111.78 (C-6'), 111.20 (C-5'), 55.93 (OCH_3), 55.81 (OCH_3), 35.40 (C-1), 33.64 (C-4), 31.13 (C-2), 28.53 (C-3); MS (EI) calcd for $C_{14}H_{20}O_2$ 220.1463, found 220.1462 (M^+, 31), 189.1272 (M-OCH_3, 1), 177.0915 (M-C_3H_7, 19), 151.0760 (M-C_5H_9, 100), 121.0654 (12), 91.0550 ($C_7H_7^+$, 13), 77.0389 ($C_6H_5^+$, 12); Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15. Found: C, 76.56; H, 9.25.



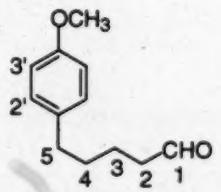
4-*tert*-Butyl-1,2-quinone (127). Silver carbonate on Celite (**124**) (50 g, 1.1 mmol) that had been under high vacuum overnight was azeotropically dried by

distillation of benzene (200 mL). When most of the benzene was evaporated, dichloromethane (150 mL) was added, followed by 4-*tert*-butylcatechol (0.05 g, 0.3 mmol), and the reaction mixture was stirred at r.t. for 4 hours. The black suspension obtained was vacuum filtered and the filtrate was evaporated *in vacuo*, affording the product **127** as a yellow solid (R_f 0.43 with 50% diethyl ether in petroleum ether, 0.05 g, quant.). IR (CH_2Cl_2 solution) 2971 (m), 1689 (m), 1666 (s) cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2:C_6D_6 1:1) δ 7.20 (dd, 1H, J = 10.4, 2.4 Hz, H-5), 6.35 (dd, 1H, J = 10.4, 0.7 Hz, H-6), 6.25 (dd, 1H, J = 2.4, 0.7 Hz, H-3), 1.20 (s, 9H, $C(C\underline{H}_3)_3$); ^{13}C NMR (75 MHz, CD_2Cl_2:C_6D_6 1:1) δ 180.85 (br, C-1, C-2), 162.52 (C-5), 140.51 (C-6), 129.74 (C-3), 124.21 (C-4), 35.91 ($\underline{C}(CH_3)_3$), 27.95 ($C(\underline{C}H_3)_3$); MS (EI) calcd for $C_{10}H_{12}O_2$ 164.0837, found 164.0840 (M^+, 68), 151.0766 ($C_9H_{11}O_2^+$, 13), 136.0887 (M-CO, 12), 121.0658 (M-C_2H_3O, 32), 93.0706 ($C_7H_9^+$, 100), 91.0549 ($C_7H_7^+$, 33); Anal. Calcd for $C_{10}H_{12}O_2$: C, 73.15; H, 7.37. Found: C, 72.86; H, 7.70.



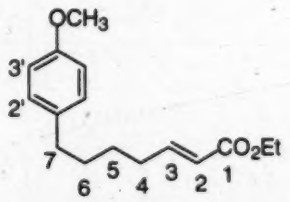
1-(3,4-Dihydroxyphenyl)-5*E*,6-octadiene (131). Compound **138** (0.088 g, 0.36 mmol) and sodium thiomethoxide (0.6 g, 8.6 mmol) were dissolved in DMF (6 mL) and the mixture was heated at 100 °C for 2.5 days. Sodium thiomethoxide (0.2 g, 2.86 mmol) again was added and the mixture was heated at 100 °C for two more days. The mixture was cooled down and acidified to pH 2 before being extracted with diethyl ether (3 × 15 mL). The combined organic layers were washed with water (40 mL), with 0.01M silver nitrate (40 mL, filtration required), with water again (40 mL), and with brine (40 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*.

Purification by silica gel column chromatography (20 to 80% diethyl ether in petroleum ether, R_f 0.30) afforded a mixture of deprotected **131** and monoprotected product (total 6 mg, 8%). Further purification by normal or reverse phase chromatography led to decomposition of the product. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.38 (d, 1H, J = 8.2 Hz, H-5'), 6.73 (br d, 1H, J = 1.6 Hz, H-2'), 6.65 (br dd, 1H, J = 8.2, 1.6 Hz, H-6'), 6.31 (ddd, 1H, J = 17.1, 10.2, 10.2 Hz, H-7), 6.05 (br dd, 1H, J = 15.2, 10.2 Hz, H-6), 5.71 (m, 1H, H-5), 5.59 (s, 2H, OH), 5.08 (br d, 1H, J = 17.1 Hz, H-8*trans*), 4.94 (m, 1H, H-8*cis*), 2.52 (t, 2H, J = 7.6 Hz, H-1), 2.10 (m, 2H, H-4), 1.59 (m, 2H, H-2), 1.43 (m, 2H, H-3); ^{13}C NMR (from 500 MHz HMQC, CD_2Cl_2) δ 137.5 (C-7), 135.5 (C-5), 131.0 (C-6), 120.0 (C-6'), 114.5 (C-2'), 114.5 (C-8), 111.0 (C-5'), 35.0 (C-1), 32.0 (C-4), 31.0 (C-2), 28.5 (C-3).



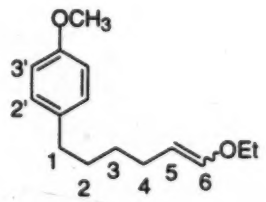
5-(4-Methoxyphenyl)pentanal (132). To a suspension of compound **118** (0.53 g, 2.8 mmol) in a 1:1 mixture of water and dioxane (88 mL) was added sodium metaperiodate (0.62 g, 2.8 mmol) and a 0.2% (w/v) solution of osmium tetroxide (12 mL of a 0.5 g osmium tetroxide in 250 mL 1:1 dioxane:water, 0.09 mmol). The reaction mixture turned from beige to orange. The mixture was stirred at r.t. overnight. The yellow mixture was filtered, and the solid collected was washed with chloroform (100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo* to afford a brown liquid. The liquid was redissolved in a 1:1 mixture of water and dioxane (80 mL) and sodium metaperiodate (0.62 g, 3 mmol) was added. The mixture was stirred at r.t. overnight. The reaction mixture was extracted twice with chloroform

and the combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. Purification by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.53 with 50%) afforded the known[188] product **132** as a yellow oil (0.41 g, 76%). IR ($CHCl_3$ cast) 2934 (m), 1723 (s), 1612 (m), 1513 (s), 1463 (m), 1245 (s), 1177 (m), 1035 (m), 830 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 9.75 (t, 1H, J = 1.8 Hz, H-1), AA'XX' system 7.10 (m, 2H, H-2') and 6.80 (m, 2H, H-3'), 3.80 (s, 3H, $OC\underline{H}_3$), 2.60 (t, 2H, J = 7.1 Hz, H-5), 2.45 (dt, 2H, J = 6.9, 1.8 Hz, H-2), 1.65 (m, 4H, H-3, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.51 (C-1), 157.83 (C-4'), 134.02 (C-1'), 129.23 (C-2'), 113.80 (C-3'), 55.25 ($O\underline{C}H_3$), 43.75 (C-2), 34.69 (C-5), 31.08 (C-4), 21.62 (C-3); MS (EI) calcd for $C_{12}H_{16}O_2$ 192.1150, found 192.1149 (M^+, 19), 147.0809 (M-OEt, 3), 121.0655 (M-C_4H_7O, 100), 107.0494 ($C_7H_7O^+$, 1), 91.0548 ($C_7H_7^+$, 5), 77.0390 ($C_6H_5^+$, 5); Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.69; H, 8.46.



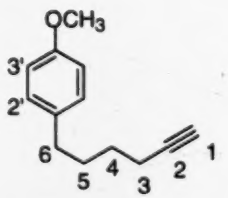
Ethyl 7-(4-methoxyphenyl)-2*E*-hexenoate (133). A solution of the aldehyde **132** (0.1 g, 0.52 mmol) in benzene (20 mL) was heated under reflux, while a solution of the compound (carboethoxymethylene)triphenylphosphorane (0.19 g, 0.55 mmol) in benzene (25 mL) was added. The mixture was heated under reflux for 4 hours then stirred at r.t. overnight. (Carboethoxymethylene)triphenylphosphorane (0.19 g, 0.55 mmol) was again added and the mixture was heated under reflux for 3 hours. The mixture was cooled to r.t., concentrated *in vacuo*, and purified by silica gel flash chromatography (9% diethyl ether in petroleum ether, R_f 0.34 with 13%). The product

133 was obtained as a colorless oil (0.15 g, 97%). IR ($CDCl_3$ cast) 2933 (m), 1719 (s), 1513 (s), 1246 (s), 1178 (m), 10 39 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.80 (m, 2H, H-3'), 6.95 (td, 1H, *J* = 15.6, 7.2 Hz, H-3), 5.80 (td, 1H, *J* = 15.6, 1.6 Hz, H-2), 4.15 (q, 2H, *J* = 7.1 Hz, $CO_2C\underline{H}_2CH_3$), 3.75 (s, 3H, $OC\underline{H}_3$), 2.50 (t, 2H, *J* = 7.3 Hz, H-7), 2.20 (dq, 2H, *J* = 7.2, 1.6 Hz, H-4), 1.60 (m, 2H, H-6), 1.50 (m, 2H, H-5), 1.25 (t, 3H, *J* = 7.1 Hz, $CO_2CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.79 (C-1), 157.74 (C-4'), 149.15 (C-3), 134.32 (C-1'), 129.25 (C-2'), 121.40 (C-2), 113.74 (C-3'), 60.16 ($CO_2\underline{C}H_2CH_3$), 55.25 ($O\underline{C}H_3$), 34.74 (C-7), 32.06 (C-4), 31.14 (C-6), 27.54 (C-5), 14.26 ($CO_2CH_2\underline{C}H_3$); MS (EI) calcd for $C_{16}H_{22}O_3$ 262.1569, found 262.1565 (M^+, 24), 188.1199 (M-$C_3H_6O_2$, 15), 174.1042 (M-$C_4H_8O_2$, 11), 147.0810 (M-$C_6H_{11}O_2$, 24); 121.0653 ($C_8H_9O^+$, 100); Anal. Calcd for $C_{16}H_{22}O_3$: C, 73.25; H, 8.45. Found: C, 72.80; H, 8.66.



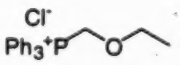
6-Ethoxy-1-(4-methoxyphenyl)-5-hexene (134). A modification of the method of Trost and Verhoeven was used.[179] To a solution of the phosphonium salt **136**[180] (1.27 g, 3.26 mmol) in diethyl ether (10 mL) cooled in an ice bath was added, very slowly, *tert*-BuLi (1.7 M in pentane, 2.2 mL, 3.6 mmol). The reaction mixture was stirred at r.t. for 15 min, then cooled to 0 °C. A solution of the aldehyde **132** (0.3 g, 1.56 mmol) in diethyl ether (3 mL) was added dropwise. The mixture was stirred at 0 °C for 30 min, then warmed to r.t., and the mixture was partitioned between diethyl ether and a saturated aqueous solution of ammonium chloride. The aqueous layer was extracted with diethyl ether (4 × 60 mL) and the combined organic layers were washed with brine

(200 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was resuspended in pentane and sodium sulfate again was added. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.54 with 25%) to give a clear oil containing a 60:40 mixture of *trans*:*cis* products respectively (0.16 g, 43%). The NMR data are for the *trans* isomer only. IR (CH_2Cl_2 cast) 2930 (m), 1512 (s), 1246 (s), 1113 (m), 1038 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.85 (m, 2H, H-3'), 6.20 (br d, 1H, J = 12.6 Hz, H-6), 4.75 (td, 1H, J = 12.6, 7.4 Hz, H-5), 3.80 (s, 3H, $OC\underline{H}_3$), 3.7 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.55 (m, 2H, H-1), 1.90 (q, 2H, J = 7.4 Hz, H-4), 1.60 (m, 2H, H-2), 1.35 (m, 2H, H-3), 1.25 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.66 (C-4'), 146.04 (C-6), 134.88 (C-1'), 129.24 (C-2'), 113.68 (C-3'), 104.13 (C-5), 64.63 ($O\underline{C}H_2CH_3$), 55.24 ($O\underline{C}H_3$), 34.87 (C-1), 31.05 (C-4), 30.30 (C-2), 27.61 (C-3), 14.78 ($CH_2\underline{C}H_3$); MS (EI) calcd for $C_{15}H_{22}O_2$ 234.1620, found 234.1620 (M^+, 43), 188.1204 (M-EtOH, 9), 160.0894 (M- EtOEt, 17), 147.0814 (M-$C_5H_{11}O$, 30), 121.0654 ($C_8H_9O^+$, 100).



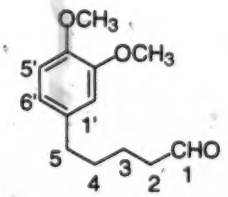
6-(4-Methoxyphenyl)-1-hexyne (135). A modification of the method of Gilbert *et al.* was used.[163] To a solution of phenyl lithium (1.8 M in diethyl ether/cyclohexane, 0.33 mL, 0.59 mmol) in THF (3 mL) cooled to -55 °C was added dropwise trimethylsilyl diazomethane (2 M in hexane, 0.28 mL, 0.56 mmol). The mixture was stirred at -55 °C for 10 min, then a solution of the aldehyde **132** (0.099 g, 0.52 mmol) in THF (1.5 mL) was added dropwise. The mixture was stirred at -55 °C for 19 hours then

at r.t. for 1.5 hours. Water (25 mL) was added to quench the reaction. The mixture was extracted with dichloromethane (3 × 40 mL) and the combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 20% diethyl ether in petroleum ether, R_f 0.85 with 50% or 0.71 with 20%) to give the alkyne **135** as a white solid (14 mg, 22%). IR ($CHCl_3$ cast) 3293 (m), 2936 (m), 2100 (w), 1512 (s), 1245 (s), 1177 (m), 1036 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ AA'XX' system 7.10 (m, 2H, H-2') and 6.85 (m, 2H, H-3'), 3.75 (s, 3H, $OC\underline{H}_3$), 2.60 (t, 2H, J = 7.4 Hz, H-6), 2.20 (dt, 2H, J = 7.1, 2.6 Hz, H-3), 1.95 (t, 1H, J = 2.6 Hz, H-1), 1.70 (m, 2H, H-5), 1.55 (m, 2H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.76 (C-4'), 134.33 (C-1'), 129.24 (C-2'), 113.75 (C-3'), 84.45 (C-2), 68.25 (C-1), 55.26 ($O\underline{C}H_3$), 34.43 (C-6), 30.64 (C-3), 27.95 (C-5), 18.28 (C-4); MS (EI) calcd for $C_{13}H_{16}O$ 188.1201, found 188.1201 (M^+, 38), 173.0965 (M-Me, 11), 160.0887 (M-C_2H_4, 14), 147.0810 (M-C_3H_5, 15), 121.0656 ($C_8H_9O^+$, 100), 91.0548 ($C_7H_7^+$, 10), 77.0391 ($C_6H_5^+$, 10).



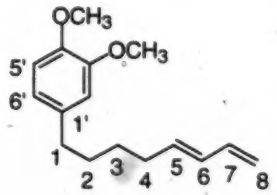
(Ethoxymethylene)triphenylphosphonium chloride (136). To a solution of triphenylphosphine (32.4 g, 0.124 mmol) in toluene (ca. 100 mL) was added chloromethylethyl ether (9.8 mL, 0.106 mmol). The reaction mixture was heated under reflux for two hours. The mixture was cooled to r.t., and the precipitate that formed was collected by vacuum filtration, washed with hexanes and allowed to dry under vacuum, affording **136** as a white solid (29 g, 77%). IR (microscope) 2988 (m), 2977 (m), 2848 (m), 2797 (m), 1586 (m), 1487 (s), 1438 (s), 1111 (s), 998 (m), 763 (s), 750 (s), 721 (m), 691 (m) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.95-7.70 (m, 15H, Ar), 5.45 (d, 2H, J = 4.9 Hz, $PC\underline{H}_2O$), 3.80 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 1.20 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, CD_3OD) δ 136.70, 136.67 (para-Ar), 135.32, 135.19

(*ortho*-Ar), 131.63, 131.46 (meta-Ar), 118.31 (d, J = 86.3 Hz, Ar$\underline{C}$P), 71.78 (d, J = 12.5 Hz, O$\underline{C}$H$_2$CH$_3$), 64.86 (d, J = 69.9 Hz, P$\underline{C}$H$_2$O), 15.08 (OCH$_2$$\underline{C}H_3$); MS (ES) calcd for $C_{21}H_{22}OP^+$ 321.1408, found 321.1410 (M^+); Anal. Calcd for $C_{21}H_{22}ClOP$: C, 70.69; H, 6.21. Found: C, 70.76; H, 6.15.



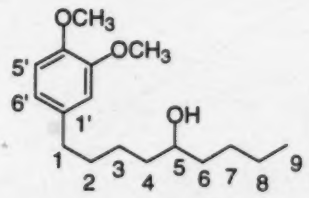
5-(3,4-Dimethoxyphenyl)pentanal (137). A modification of the procedure of Pappo *et al.*[159] was used to prepare the known[189] compound **137**. To a solution of **123** (0.05 g, 0.23 mmol) in a 1:1 mixture of dioxane:water (2 mL) was added osmium tetroxide (1 mL of a 0.5 g osmium tetroxide solution in 250 mL 1:1 dioxane:water, 0.009 mmol). After the mixture had stirred for 10 min at r.t., sodium metaperiodate (0.5 g, 2.34 mmol) was added over 20 min. The mixture was then stirred at r.t. for one hour and osmium tetroxide again was added (2 mL of a 0.5 g osmium tetroxide solution in 250 mL 1:1 dioxane:water, 0.018 mmol). The reaction mixture was allowed to stir at r.t. overnight. At which time an additional amount of sodium metaperiodate was added (0.3 g, 1.4 mmol) and the mixture was stirred for one hour. The reaction mixture was diluted with diethyl ether (15 mL) and filtered. The aqueous layer was extracted with diethyl ether (3 × 15 mL), and the combined organic layers were washed with brine (40 mL), dried (Na_2SO_4), and evaporated *in vacuo*. Purification by silica gel column chromatography (33% diethyl ether in petroleum ether, R_f 0.30 with 50%) afforded **137** (0.04 g, 75%). IR (CH_2Cl_2 cast) 2936 (m), 1722 (s), 1516 (s), 1261 (s), 1235 (s), 1029 (s), 808 (w), 764 (w) cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.75 (t, 1H, J = 1.7 Hz, H-1), 6.80 (d, 1H, J = 8.6 Hz, H-5'), 6.70 (m, 2H, H-2', H-6'), 3.85 (s, 3H, OC$\underline{H}_3$), 3.80 (s,

3H, $OC\underline{H}_3$), 2.55 (m, 2H, H-5), 2.45 (m, 2H, H-2), 1.65 (m, 4H, H-3, H-4); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 202.84 (C-1), 149.51 (C-3'), 147.83 (C-4'), 135.29 (C-1'), 120.56 (C-2'), 112.50 (C-6'), 112.01 (C-5'), 56.26 ($O\underline{C}H_3$), 56.13 ($O\underline{C}H_3$), 44.14 (C-2), 35.58 (C-5), 31.49 (C-3), 22.10 (C-4); MS (EI) calcd for $C_{13}H_{18}O_3$ 222.1256, found 222.1258 (M^+, 55), 194.1316 (M-CO, 6), 177.0918 (M-OEt, 3), 164.0836 (M-C_3H_6O, 4), 151.0757 (M-C_4H_8O, 100), 107.0495 ($C_7H_7O^+$, 12), 91.05457 ($C_7H_7^+$, 11), 77.0392 ($C_6H_5^+$, 11); Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.17; H, 8.31.



1-(3,4-Dimethoxyphenyl)-5*E*,6-octadiene (138). A suspension of sodium hydride (0.32 g of a 50% oil dispersion, prewashed with hexane and with diethyl ether, 6.6 mmol) in dimethyl sulfoxide (1.6 mL, 23 mmol) was heated at 80 °C for one hour. This mixture was cooled to r.t., at which point a solution of methyltriphenylphosphonium bromide (0.75 g, 2.1 mmol) in dimethyl sulfoxide (6 mL) was added, and the mixture was stirred at r.t. for 15 min. A solution of aldehyde **140** (0.2 g, 0.81 mmol) in dimethyl sulfoxide (4 mL) was then slowly added. The reaction mixture was stirred at r.t. for 6 hours, then quenched with water (10 mL) and acidified to pH 3 (1N HCl). The mixture was extracted with diethyl ether (3 × 25 mL) and the combined organic layers were washed with brine (80 mL), dried (Na_2SO_4), and evaporated *in vacuo*. Purification by silica gel column chromatography (33% diethyl ether in petroleum ether, R_f 0.39) afforded the product **138** (0.11 g, 56%). IR (CH_2Cl_2 cast) 2931 (m), 1515 (s), 1464 (m), 1262 (s), 1236 (s), 1031 (m), 898 (w), 804 (w) cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 6.80 (d, 1H, J = 8.7 Hz, H-5'), 6.70 (m, 2H, H-2', H-6'),

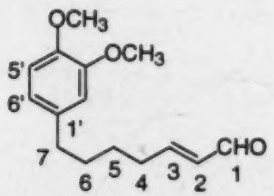
6.30 (td, 1H, J = 16.9, 10.3 Hz, H-7), 6.05 (dd, 1H, J = 15.0, 10.3 Hz, H-6), 5.70 (m, 1H, H-5), 5.10 (d, 1H, J = 16.9 Hz, H-8*trans*), 4.95 (d, 1H, J = 10.3 Hz, H-8*cis*), 3.82 (s, 3H, OC$\underline{H}_3$), 2.78 (s, 3H, OC$\underline{H}_3$), 2.55 (t, 2H, J = 7.5 Hz, H-1), 2.10 (q, 2H, J = 7.2 Hz, H-4), 1.60 (m, 2H, H-2), 1.45 (m, 2H, H-3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 149.45 (C-3'), 147.71 (C-4'), 137.81 (C-7), 135.87 (C-5), 135.80 (C-1'), 131.42 (C-6), 120.54 (C-2'), 114.82 (C-8), 112.55 (C-6'), 111.98 (C-5'), 56.27 (O$\underline{C}H_3$), 56.11 (O$\underline{C}H_3$), 35.69 (C-1), 32.79 (C-4), 31.65 (C-2), 29.22 (C-3); MS (EI) calcd for $C_{16}H_{22}O_2$ 246.1620, found 246.1621 (M^+, 47), 220.1462 (M-C_2H_2, 18), 177.0915 (M-C_5H_9, 32), 164.0836 (M-C_6H_9, 40), 151.0760 (M-C_7H_{11}, 100), 107.0496 ($C_7H_7O^+$, 15), 91.0547 ($C_7H_7^+$, 13), 77.0393 ($C_6H_5^+$, 14); Anal. Calcd for $C_{16}H_{22}O_2$: C, 76.33; H, 9.15. Found: C, 75.66; H, 9.34.



Alternative method for the preparation of 1-(3,4-dimethoxyphenyl)-5*E*,6-octadiene (138) leading to 1-(3,4-dimethoxyphenyl)-5-hydroxynonane (139) as the major product. The Schlosser modification was used to optimize the yield of *trans* product.[164] To a suspension of allyl triphenylphosphonium bromide (0.09 g, 0.225 mmol) in THF (5 mL) cooled to -70 °C, was slowly added *n*-BuLi (0.20 mL of 2.2 M in hexanes, 0.42 mmol). The yellow mixture was stirred at -40 °C for three hours, by which time it had turned red. Aldehyde **137** (0.05 g, 0.225 mmol) was added and the mixture was stirred at -40 °C for one hour. The mixture was then cooled to -70 °C and *n*-BuLi (0.12 mL, 0.25 mmol) was again added, and the reaction was kept at that temperature for one hour. After isopropanol (0.05 mL) was added, the mixture was

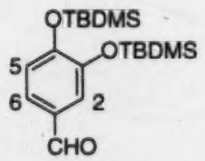
warmed to r.t., and water (10 mL) was added to quench the reaction. The products were extracted in diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and evaporated *in vacuo*. Purification of the residue by silica gel flash column chromatography (50% diethyl ether in petroleum ether), afforded the the desired diene **138** (R_f 0.71, 6 mg, 10%), and the side product **139** (R_f 0.29, 36 mg, 55%).

Data for 1-(3,4-dimethoxyphenyl)-5-hydroxynon-1-ane (139). IR (microscope) 2927 (s), 2854 (s), 1515 (s), 1465 (s), 1258 (s), 1236 (s), 1155 (s), 1136 (s), 1029 (s) cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 6.78 (m, 1H, H-5'), 6.70 (m, 2H, H-2', H-6'), 3.92 (s, 3H, $OC\underline{H}_3$), 3.96 (s, 3H, $OC\underline{H}_3$), 3.55 (m, 1H, H-5), 2.56 (t, 2H, J = 7.6 Hz, H-1), 1.60 (m, 2H, H-2), 1.50-1.22 (m, 11H, H-3, H-4, H-6, H-7, H-8, OH), 0.92 (t, 3H, J = 6.8 Hz, H-9); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 149.46 (C-3'), 147.72 (C-4'), 135.96 (C-1'), 120.55 (C-2'), 112.59 (C-6'), 112.02 (C-5'), 72.11 (C-5), 56.27 ($O\underline{C}H_3$), 56.13 ($O\underline{C}H_3$), 37.80, 37.71 (C-4, C-6), 35.86 (C-1), 32.20 (C-3), 28.29 (C-7), 25.75 (C-8), 23.20 (C-2), 14.27 (C-9); MS (EI) calcd for $C_{17}H_{28}O_3$ 280.2039, found 280.2040 (M^+, 81), 262.1930 (M-H_2O, 15), 177.0916 (M-$C_6H_{15}O$, 25), 151.0759 ($C_9H_{11}O_2^+$, 100); MS (ES) 863 ($3MNa^+$, 6), 583 ($2MNa^+$, 45), 303 (MNa^+, 100).



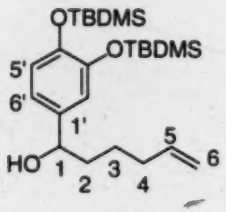
7-(3,4-Dimethoxyphenyl)-2-heptenal (140). The aldehyde **137** (0.34g, 1.5 mmol) and formylmethylenetriphenylphosphorane (1.4 g, 4.6 mmol, recrystallized from acetone) were dissolved in benzene (80 mL) and heated under reflux for two days. The reaction was followed by IR (ν_{satCHO}= 1720 cm^{-1}, $\nu_{unsatCHO}$ = 1686 cm^{-1}).

Formylmethylenetriphenylphosphorane (0.2 g, 0.66 mmol) was again added and the mixture was heated under reflux for one more day. The reaction mixture was filtered through a plug of silica gel and washed with diethyl ether. The filtrate was evaporated *in vacuo*. Purification by silica gel column chromatography (25% diethyl ether in petroleum ether, R_f 0.29 with 50%) afforded the product **140** as a yellow oil (0.27 g, 73%). IR (CH_2Cl_2 cast) 2933 (m), 1687 (s), 1515 (s), 1261 (s), 1235 (s), 1156 (s), 1029 (s) cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 9.50 (d, 1H, J = 7.9 Hz, H-1), 6.85 (td, 1H, J = 15.6, 6.8 Hz, H-3), 6.80 (d, 1H, J = 8.7 Hz, H-5'), 6.70 (m, 2H, H-2', H-6'), 6.10 (tdd, 1H, J = 15.6, 7.9, 1.4 Hz, H-2), 3,80 (s, 3H, $OC\underline{H}_3$), 3.75 (s, 3H, $OC\underline{H}_3$), 2.60 (t, 2H, J = 7.3 Hz, H-7), 2.35 (dq, 2H, J = 7.3, 1.4 Hz, H-4), 1.7-1.4 (m, 4H, H-5, H-6); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.28 (C-1), 159.06 (C-3'), 149.50 (C-4'), 147.83 (C-3), 135.40 (C-1'), 133.38 (C-2), 120.56 (C-2'), 112.51 (C-6'), 112.01 (C-5'), 56.26 ($O\underline{C}H_3$), 56.14 ($O\underline{C}H_3$), 35.56 (C-7), 33.00 (C-4), 31.54 (C-6), 27.86 (C-5); MS (EI) calcd for $C_{15}H_{20}O_3$ 248.1413, found 248.1417 (M^+, 27), 222.1259 (M-C_2H_4, 10), 177.0915 (M-C_4H_7O, 9), 151.0760 (M-$C_6H_{11}O$, 100), 121.0652 ($C_8H_9O^+$, 5), 107.0495 ($C_7H_7O^+$, 8), 91.0546 ($C_7H_7^+$, 6), 77.0389 ($C_6H_5^+$, 6); Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 71.54; H, 8.15.



3,4-Di-*tert*-butyldimethylsilyloxybenzaldehyde (141). The known[190] compound **141** was prepared as follows. 3,4-Dihydroxybenzaldehyde (1.0 g, 7.2 mmol), *tert*-butyldimethylsilylchloride (4.3 g, 28.8 mmol) and imidazole (2.0 g, 29.4 mmol) were dissolved in DMF (10 mL). The reaction mixture was stirred at r.t. for 4 hours, then poured into hexanes (100 mL) and washed with water (3 × 100 mL). The

organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. Purification by silica gel column chromatography (9% diethyl ether in petroleum ether, R_f 0.54 with 20% and 0.52 with 12.5%) afforded the product **141** as a colorless oil (0.87 g, 33%). IR ($CDCl_3$ cast) 2931 (s), 2859 (m), 1697 (s), 1505 (s), 1295 (s), 1255 (m), 902 (s), 841 (s), 783 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.82 (s, 1H, CHO), 7.34 (d, 1H, J = 8.0 Hz, H-6), 7.34 (m, 1H, H-2), 6.92 (d, 1H, J = 8.0 Hz, H-5), 0.98 (m, 18H, $SiC(C\underline{H}_3)_3$), 0.22 (s, 3H, $SiC\underline{H}_3$), 0.21 (s, 3H, $SiC\underline{H}_3$); ^{13}C NMR (from 500MHz HMQC, $CDCl_3$) δ 191 (CHO), 131 (C-1), 125, 120 (C-2, C-6), 121 (C-5), 25 ($SiC(\underline{C}H_3)_3$), 9 ($Si(\underline{C}H_3)_2$); MS (EI) calcd for $C_{19}H_{34}O_3Si_2$ 366.2047, found 366.2044 (M^+, 1), 309.1341 (M-C_4H_9, 93), 73.0474 (Me_3Si^+, 100); Anal. Calcd for $C_{19}H_{34}O_3Si_2$: C, 62.24; H, 9.35. Found: C, 62.25; H, 9.53.



1-(3,4-Di-*tert*-butyldimethylsilyloxyphenyl)-5-hexen-1-ol (142). The procedure used for the preparation of 1-(4-methoxyphenyl)-4-penten-1-ol (**114**) was adopted. Thus, compound **141** (0.75 g, 2.1 mmol) was added to 5-bromo-1-pentene (**112**) (0.28 mL, 2.4 mmol) previously treated with magnesium turnings (0.08 g, 3.3 mmol) in THF. Purification by silica gel column chromatography (9% diethyl ether in petroleum ether, R_f 0.34 with 17%) afforded the product **142** as a viscous colorless oil (0.52 g, 60%). IR (CD_2Cl_2 cast) 3347 (br m), 2931 (s), 2897 (m), 2859 (s), 1472 (m), 1295 (s), 1254 (s), 1222 (m), 909 (s), 838 (s), 781 (s) cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 6.80 (m, 3H, H-2', H-5', H-6'), 5.80 (m, 1H, H-5), 5.00 (ddd, 1H, J = 17.2, 10.6, 1.6 Hz, H-6*trans*), 4.95 (ddd, 1H, J = 10.1, 2.2, 1.6 Hz, H-6*cis*), 4.55 (dt, 1H, J = 6.7, 3.4

Hz, H-1), 2.05 (tq, 2H, J = 6.7, 1.0 Hz, H-2), 1.80 (br s, 1H, J = 3.4 Hz, OH), 1.65 (m, 2H, H-4), 1.40 (m, 2H, H-3), 1.00 (s, 18H, $SiC(C\underline{H}_3)_3$), 0.20 (s, 12H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 147.14 (C-3'), 146.54 (C-4'), 139.25 (C-5), 138.86 (C-1'), 121.19 (C-2'), 119.30 (C-6), 119.23 (C-6'), 114.66 (C-5'), 74.26 (C-1), 38.91 (C-4), 34.00 (C-2), 26.16 ($SiC(\underline{C}H_3)_3$), 26.15 ($SiC(\underline{C}H_3)_3$), 25.53 (C-3), 18.77 ($Si\underline{C}(CH_3)_3$), -3.96 ($Si\underline{C}H_3$), -3.90 ($Si\underline{C}H_3$); MS (EI) calcd for $C_{24}H_{44}O_3Si_2$ 436.2829, found 436.2826 (M^+, 15), 379.2123 (M-C_4H_9, 9), 247.1153 (M-$C_{10}H_{25}OSi$, 39), 73.0473 (Me_3Si^+, 100); Anal. Calcd for $C_{24}H_{44}O_3Si_2$: C, 66.00; H, 10.15. Found: C, 65.98; H, 10.44.

3. Fermentation and Incorporation Methods

Fermentation of *Penicillium aurantiogriseum*, isolation of compactin (5) and ML-236 (25), and growth curve. All solutions, glassware and tools were autoclaved. The cultures of *Penicillium aurantiogriseum* ATCC 60762 (American Type Culture Collection, Rockville, MD, a gift from Horace G. Cutler, Agricultural Research Service, Athens, GA; UAMH 7434 and 7558) were maintained on slants made from 5 g of Bacto-agar, 25 g of Pablum, and 250 mL of water. The inoculated slants were incubated at 28 °C for 8 days in the dark, after which they were stored at 4 °C. The trace elements solution required for the growth medium was kept at 4 °C and was prepared from: 19 mg of $(NH_3)_6Mo_7O_{24}.4H_2O$, 25 mg of $CuCl_2.2H_2O$, 56 mg of H_3BO_3, 76 mg of $CaCl_2$, 200 mg of $ZnSO_4.7H_2O$, 0.67 g of $MnSO_4.H_2O$, and 1 g of $FeSO_4.7H_2O$, in 1 L of water. The growth medium (GM) contained: 0.25 g of corn steep solids, 1.0 g of Pablum, 1.5 g of glucose, 2.0 mL of trace elements solution, and 4.2 g of tomato paste, in 100 mL of water. The required production medium (PM) contained: 24.5 g of succinic acid at pH 4.0, 2 g of peptone, 10 g of glucose, and 60 g of malt extract, in 2 L of water. The GM (100 mL in 500 mL Erlenmeyer) was inoculated with one *P.*

aurantiogriseum slant and fermented at 28 °C and 200 rpm for two days. The GM culture (12 × 10 mL) was used to inoculate the PM (12 × 140 mL in a 500 mL Erlenmeyer) which were grown at 28 °C and 200 rpm for 7 days. Compactin (**5**) and ML-236C (**25**) were isolated and purified as follows. The PM cultures were vacuum filtered through two filter papers, and the resulting broth was acidified to pH 2 (2N HCl), saturated with NaCl, and extracted with ethyl acetate (4 × 150 mL). The combined mycelia was blended with water (150 mL) at low speed for 30 sec, acidified to pH 2 (2N HCl), and blended with ethyl acetate (400 mL) for 1 min, and then filtered through a bed of Celite. The filtrate was further extracted with ethyl acetate (300 mL) and all the combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. In order to ensure that all the compactin (**5**) and ML-236C (**25**) were lactonized, toluene (100 mL) was added to the residue (280 mg) and the solution was heated under reflux with a sohxlet containing calcium hydride for 90 min. The toluene was removed *in vacuo* and compactin (**5**) and ML-236C (**25**) were pre-purified using silica gel column chromatography (30 to 70% ethyl acetate in hexanes, R_f 0.35 with 30%). The final purification was achieved by HPLC using a μBondapak radial compression reverse phase C_8, 25 × 100 mm, 15-20 μm, 300 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min. The gradient was kept at 40% acetonitrile in water and 0.1% trifluoroacetic acid (TFA) for 10 min, then raised from 40% to 49% over 11 min, kept at 49% for 8 min, raised from 49% to 56% over 8 min, from 56% to 95% over 2 min, and kept at 95% for 9 min. The UV detector was set at 238 nm. The retention times were 37 min for compactin (**5**) and 28 min for ML-236C (**25**). Compactin (**5**) was obtained as a white solid (100 mg/L of cultures) and ML-236C (**25**) was a beige solid (35 mg/L).

The growth curve of *P. aurantiogriseum* was prepared by quantifying both the wet mycelia collected by filtration of the cultures, and the amount of compactin (**5**) present in both the filtrate and the mycelia, twice daily for nine days. Each PM culture

was extracted as above. The compactin (**5**) contained in the residue from the toluene reflux was quantified by analytical HPLC against a calibration curve prepared from lovastatin (**1**) standard solutions. The column used was a Novapak radial compression reverse phase C_{18}, 8 × 100 mm, 4 μm (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 40% acetonitrile in water and 0.1% TFA for 5 min, then went from 40% to 52% over 20 min, and from 52% to 95% over 5 min. The UV detector was set at 220 nm. The retention times were 24.1 min for compactin (**5**) and 29.2 min for lovastatin (**1**).

Incorporation studies of [2,3-$^{13}C_2$]-acetoacetic acid, *N*-acetylcysteamine thioester (28a) into compactin (5) using *Penicillium aurantiogriseum*. The diketide [2,3-$^{13}C_2$]-acetoacetic acid, *N*-acetylcysteamine thioester (**28a**) was prepared by Dr. Yaoquan Liu from our research group as described in his thesis.[97] The diketide solution used for feeding contained 32.8 mg of **28a** in 1.2 mL of absolute ethanol. The β-oxidation inhibitor solution contained 31.2 mg of ethyl 3-hydroxypent-4-ynoate (**26**) in 1.2 mL of absolute ethanol. Different methods were attempted and they all utilize the media GM and PM described for the isolation of compactin (**5**).

The replacement medium (RM) used in the first method contained: 100 g of glucose, 1 g of Na_2HPO_4, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of KCl, and 0.01 g of $FeSO_4.7H_2O$, in 1 L of water. After two days of growth in the PM (2 × 150 mL) the cultures were filtered through cheesecloth, rinsed with RM, and the mycelia gently transferred into fresh RM (2 × 150 mL). Feeding was started after the transfer and performed every 12 hours for three days (0.2 mL/150 mL of culture twice daily of each the diketide **28a** and the inhibitor **26** solutions). The cultures were incubated for one more day following the last addtion of diketide **28a** and inhibitor **26**. Work up and purification of compactin (**5**) were accomplished as described above. The yield of

compactin (**5**) was found to be greatly reduced (3.3 mg/L), compared to growth in PM alone.

To check if the decrease of compactin (**5**) production was due to the RM or to the transfer itself, the experiment was repeated in the same way but the filtered mycelia were transferred into fresh PM instead of RM. Once again, the yield of compactin was greatly reduced (8.0 mg/L).

In order to make sure that the production of compactin was not affected by the β-oxidation inhibitor, the cultures were grown in the exact same way as during the first isolation of compactin (**5**) except that the inhibitor **26** solution was fed to the cultures starting after two days of growth in PM. The production of compactin (**5**) was found to be back to normal (100 mg/L) despite the inhibitor. Therefore, the final method used did not involve any filtration of the PM.

The GM (100 mL in 500 mL Erlenmeyer) was inoculated with one *P. aurantiogriseum* slant and incubated at 28 °C and 200 rpm for two days. The GM cultures (10 mL) were used to inoculate the PM (140 mL in a 500 mL Erlenmeyer) which were then fermented at 28 °C and 200 rpm for 7 days. The feeding of diketide **28a** and inhibitor **26** was started two days after the mycelia had been growing in PM. Every 8 hours additional diketide **28a** solution (0.12 mL), and inhibitor **26** solution (0.12 mL) were added to the cultures over 4 days. The cultures were grown for another day and compactin (**5**)was purified in the same way as for its original isolation. According to the ^{13}C NMR no intact incorporation of the diketide **28a** occurred, very likely due to rapid degradation (scrambling of the ^{13}C atoms was observed).

***In vivo* incorporation studies of [2,3-$^{13}C_2$]-3-hydroxybutyric acid, *N*-acetylcysteamine thioester (29a) into compactin (5) using *Penicillium aurantiogriseum*.** The diketide solution used for feeding contained 29.8 mg of [2,3-$^{13}C_2$]-3-hydroxybutyric acid, *N*-acetylcysteamine thioester (**29a**) in 1.2 mL of absolute

ethanol. The β-oxidation inhibitor solution contained 29.8 mg of ethyl 3-hydroxypent-4-ynoate (**26**) in 1.2 mL of absolute ethanol. Based on the fermentation experiments conducted for the incorporation of [2,3-$^{13}C_2$]-acetoacetic acid, *N*-acetylcysteamine thioester (**28a**), the only method used here did not involve the filtration of the PM. The GM (100 mL in 500 mL Erlenmeyer) was inoculated with one *P. aurantiogriseum* slant and incubated at 28 °C and 200 rpm for two days. The GM cultures (2 × 10 mL) were used to inoculate the PM (2 × 140 mL in a 500 mL Erlenmeyer) which then fermented at 28 °C and 200 rpm for 7 days. The feeding was started two days after the mycelia had been growing in PM. For 4 days, at 8 hour intervals, the diketide solution (0.12 mL), and the inhibitor solution (0.12 mL) were added to the cultures. The fungi were grown for an additional day after the last addition of diketide **29a** and inhibitor **26**. Compactin (**5**) was purified in the same way as for its original isolation. According to the ^{13}C NMR no incorporation of the intact diketide **29a** occurred, very likely because of rapid degradation (scrambling of the ^{13}C atoms was observed).

Fermentation of the *Aspergillus terreus* control and *lovE* strains, and isolation of lovastatin (1). The *Aspergillus terreus lovE* strain (UAMH 9155) was provided by Professor C. R. Hutchinson (University of Wisconsin). It contained extra copies of the lovastatin *lovE* gene expressed under the control of the *A. oryzae* glucoamylase promoter and a phleomycin resistance vector. The *A. terreus* control strain (UAMH 9156) contained only the phleomycin resistance vector. All solutions, glassware and tools were autoclaved. The cultures of *A. terreus* were maintained on agar slants made from 5 g of Bacto-agar, 25 g of Pablum, and 250 mL of water. After inoculation, the fungi were incubated at 28 °C for 4 days, after which they were stored at 4 °C. The grown slants showed both beige and white coloration. The *A. terreus lovE* strain was grown either with the same fermentation method as for *A. nidulans* or as follows. The latter method tends to give cultures that grew along the flask walls and as

a result, were very hard to filter. The fermentation medium GP9 contained: 1.2 g of Ardamine pH, 1.2 g of cerelose, 4 g of Pharmamedia, 9 g of ammonium citrate, and 245 g of lactose, dissolved in 1 L of water at pH 7.2. The GP9 (750 mL in 2 L Erlenmeyer) was inoculated with one slant of *A. terreus lovE* and fermented at 30 °C and 200 rpm for 10 days. A sterile 10% aqueous solution of soluble starch (75 mL) was added then the cultures were incubated for an extra day. Lovastatin (**1**) was isolated as described in the compounds section for **1**. When the *A. terreus lovE* strain was compared to the *A. terreus* control strain, no new metabolites were detected under the conditions used.

Fermentation of *Aspergillus terreus* XS-1P, quantification of lovastatin production, and ancymidol inhibition. The *Aspergillus terreus* XS-1P strain was provided by Professor C. R. Hutchinson (University of Wisconsin). *A. terreus* XS-1P (UAMH 9271) was transformed with a plasmid containing the *lovE* gene and its own promoter from the lovastatin biosynthesis gene cluster cloned into the plasmid pPLOA (phleoR).[93] It produces significantly more lovastatin than the wild type strain that it is derived from. All solutions, glassware and tools were autoclaved. The cultures of *A. terreus* XS-1P were maintained on agar slants made from 5 g of Bacto-agar, 25 g of Pablum, and 250 mL of water. After inoculation, the fungi were incubated at 28 °C for 7 days, after which they were stored at 4 °C. The required Vogel's trace elements solution was prepared from 0.2 g of $FeCl_3$, 0.04 g of $ZnCl_2$, 0.01 g of $CuCl_2$, 0.01 g of $MnCl_2.4H_2O$, 0.01 g of $Na_2B_4O_7.10H_2O$, and 0.01 g of $(NH_4)_6Mo_7O_{24}.7H_2O$, diluted in 1 L of water. The Seed Medium (SM) contained: 2 g of tomato paste, 0.5 g of oat flour, 0.5 g of glucose, 0.25 g of corn steep solid, and 0.5 mL of Vogel's trace elements solution, in 50 mL of water. The Fermentation Medium (FM) was prepared from: 45 g of glucose, 24 g of peptonized milk, 2.5 g of yeast extract, and 2.5 g of polyethylene glycol 2000, in 500 mL of water. The SM (50 mL in 500 mL Erlenmeyer) was inoculated with one *A. terreus* XS-1P slant or one *A. terreus* control (see above) slant

and fermented at 28 °C and 220-250 rpm for one day. The SM cultures (4 × 20 mL) were used to inoculate the FM (4 × 500 mL in a 2 L Erlenmeyer) which were incubated at 28 °C and 220 rpm for 5 days. In an effort to produce less oxidized derivatives of lovastatin (**1**) (e.g. 4a,5-dihydromonacolin L (**11**)) ancymidol (0.5 mL per 500 mL of cultures of a 26 mg in 15 mL of 70% ethanol solution), a known cytochrome P450 enzyme inhibitor, was added every 8 hours for the 5 days to one of the FM cultures. Lovastatin (**1**) production was monitored by isolation and purification using the method described in the compounds section for **1**. The cultures treated with ancymidol showed production of monacolin J (**14**) on TLC (90% methanol in 10% 15 mM phosphoric acid, R_f 0.72). The *A. terreus* XS-1P cultures were compared against the ancymidol inhibited cultures and the control strain. The lovastatin (**1**) production was found to be in a ratio of 30:10:1 respectively while the wet mycelia production ratio was 2.0:1.4:1.0 respectively.

Fermentation of *Aspergillus terreus* XS-1P and incorporation studies of [1,2-$^{13}C_2$]-acetyl CoA (39a) into lovastatin (1) using protoplasts. *A. terreus* XS-1P was fermented as described above for lovastatin (**1**) quantification except that the FM cultures (2 × 500 mL) were incubated only for one day. The wet mycelia (70 g) were collected by vacuum filtration through 4 layers of cheesecloth, washed with water (600 mL), and gently split into five portions. Two negative controls were prepared, and three osmotic stabilizers ($MgSO_4$, KCl, and mannitol) were investigated for the preparation of protoplasts.[102] The buffer used was 50 mM citrate-phosphate at pH 5. The enzymes used to consume the cell walls were a mixture of lysing enzymes from *Trichoderma harzianum* (400 mg, Sigma) and Novozym 234 lysing enzymes from *Trichoderma harzianum* (600 mg, Calbiochem). The negative control 1 contained mycelia (14 g) and buffer (125 mL), and was later fed with [1,2-$^{13}C_2$]-acetyl CoA (**39a**) as described below.

g, 0.7 M), and lysing enzymes (250 mg). This control was not fed with [1,2-$^{13}C_2$]-acetyl CoA (**39a**). Reaction 1 contained mycelia (14 g), buffer (125 mL), $MgSO_4$ (10.5 g, 0.7 M), and lysing enzymes (250 mg). Reaction 2 was a mixture of mycelia (14 g), buffer (125 mL), KCl (6.5 g, 0.7 M), and lysing enzymes (250 mg). Reaction 3 was prepared by mixing mycelia (14 g), buffer (125 mL), mannitol (15.9 g, 0.7 M), and lysing enzymes (250 mg). The five mixtures were left to digest at 37 °C and 100 rpm for 4 hours. The solutions were filtered separately through a Miracloth, and rinsed with FM. The 'protoplasts' were each transferred into 125 mL of fresh sterile FM. The cultures were left in the shaker at 30 °C and 150 rpm for two days. Feedings of [1,2-$^{13}C_2$]-acetyl CoA (**39a**) (1 mL of 137 mg in 10 mL of water) started after the transfer, and continued at 8 hour intervals for two days, except for the negative control 2. The cultures were fermented for two more days after the last feeding before work up. The control 1, which did not contain lysing enzymes, looked very thick compared to the other cultures. The five cultures were vacuum filtered separately through two filter papers each. The acidified filtrates were individually extracted with ethyl acetate (3 × 100 mL). The wet mycelia (ca. 6 g of skin-colored mycelia each, except blank 1 which was white) were blended separately for 30 sec with water (100 mL). After acidification to pH 1, the solution was blended with ethyl acetate (100 mL) for one minute. The combined organic layers for each assay were separately washed with brine (300 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residues (0.5-2.0 g) were redissolved in toluene (30 mL each) and heated under reflux with a soxhlet containing calcium hydride for one hour. Toluene was evaporated *in vacuo*, and the lovastatin (**1**) present in each residue (0.12-0.17 g) was isolated by HPLC for analysis of its ^{13}C content. A μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON) was used. At a flow rate of 15 mL/min, the gradient started at 30% acetonitrile in water and 0.1% TFA, then went from 30% to 40% over 10 min, from 40% to 49% over 11 min, was kept at 49% for 6 min, raised

from 49% to 100% over 3 min, and kept at 100% for 5 min. The UV detector was set at 240 nm. The retention time of lovastatin (**1**) was 20.7 min. According to NMR and MS, the lovastatin (**1**) purified (ca. 2 mg each) did not show significant incorporation of ^{13}C.

Fermentation of the *Aspergillus nidulans* control (GM32.4), *lovB*, and *lovB+lovC* strains. These strains were provided by Professor C. R. Hutchinson (University of Wisconsin).[94] *Aspergillus nidulans* GM32.4 (UAMH 8966) is a control strain. It had been tranformed with a plasmid containing a marker that encodes an enzyme necessary for the biosynthesis of uridine which the host strain lacks. The *A. nidulans lovB* strain (UAMH 8965 or WMH1738) had been transformed with a plasmid containing the uridine marker as well as the LNKS gene of the lovastatin gene cluster under the control of the *alcA* promoter. *A. nidulans lovB+lovC* (UAMH 9442 or WMH1750) refers to the *A. nidulans lovB* strain transformed with a plasmid containing the phleomycin resistance gene and *lovC* under the control of *alcA* promoter. All solutions, glassware, and tools were autoclaved. PABA stock solution (needed for growth) was prepared from: 0.1 g of *p*-aminobenzoic acid (PABA) in 100 mL of Milli-Q water. The required 10 × AMM salts solution contained: 60 g of $NaNO_3$, 5.2 g of KCl, and 15.2 g of KH_2PO_4, diluted to 1 L at pH 6.5. The trace elements solution required for production medium had: 1 g of $FeSO_4.7H_2O$, 8.8 g of $ZnSO_4.7H_2O$, 0.4 g of $CuSO_4.5H_2O$, 0.15 g of $MnSO_4.4H_2O$, 0.1 g of $Na_2B_4O_7.10H_2O$, and 0.05 g of $(NH_4)_6Mo_7O_{24}.4H_2O$, diluted to 1 L with 0.5 mL HCl added. Agar complete medium (ACM) contained: 20 g of malt extract, 1 g of Bacto-peptone, 20 g of glucose, 20 g of agar, and 1 mL of PABA stock, in 1 L of water. The ACM slants were inoculated with *A. nidulans* (transformed or wild-type control) and incubated for 5 days at 30 °C. Growth medium (YEPD) contained: 20 g of yeast extract, 1 g of Bacto-peptone, 20 g of glucose, and 1 mL of PABA stock, diluted to 1 L. This was inoculated from one slant

of *A. nidulans* and then fermented (1 L in a 2 L Erlenmeyer) at 200 rpm and 30 °C for one day. Miracloth (Calbiochem, La Jolla, CA) was used to filter the mycelia, which were washed with production medium (2 L) and then transferred into fresh production medium (1 L in a 2 L Erlenmeyer). The production medium (AMM) consisted of: 1 mL of trace elements solution, 100 mL of 10 × AMM salts solution, 1 mL of PABA stock, and 0.9 mL of cyclopentanone for induction, diluted to 1 L, then autoclaved, and finally treated with 2.5 mL of sterile 20% $MgSO_4.7H_2O$ and 25 mL of sterile 40% lactose solution. The AMM culture was incubated at 30 °C and 200 rpm. The pigments **41** and **42** were isolated after growing in the AMM for two days. 4a,5-Dihydromonacolin L (**11**) was isolated after 7 days in the AMM. The cultures were found to produce more of compounds **41** and **42** when they were fed with sodium acetate (2 mL of a 12% aqueous solution to each 1 L AMM every 4 hours for two days).

Labeling of the pigments 41a, 41b, 41c, 42a, 42b, 42c, and of 4a,5-dihydromonacolin L 11a. Because of the instability of the pigments **41** and **42**, the ^{1}H and ^{13}C NMR assignments were assisted by biosynthetic labeling with ^{13}C. For that purpose, 2 mL of a 5% aqueous solution of labeled sodium acetate (1-^{13}C, 2-^{13}C, or 1,2-$^{13}C_2$, each 99% isotopic purity, Cambridge Isotope Laboratories, Andover, MA) was added to each 1 L of production media every 4 hours for two days starting after transfer. The feeding of sodium acetate was found to increase the yield of **41a**, **41b**, **41c**, **42a**, **42b**, and **42c** from the *A. nidulans lovB* cultures. Surprisingly, the labeling by di-^{13}C-sodium acetate was as high as 20% per carbon for labeled 4a,5-dihydromonacolin L (**11a**), from NMR and MS. The labeling by mono- and di-^{13}C-sodium acetate was even higher at a rate of 70% for **41a**, **41b**, **41c**, **42a**, **42b**, and **42c**.

Isolation and purification of the pigments 41, 41a, 41b, 41c, 42, 42a, 42b, and 42c from *Aspergillus nidulans lovB*. The pigments **41**, **41a**, **41b**, **41c**, **42**, **42a**,

42b, and **42c** were isolated from the *A. nidulans lovB* cultures as follows. Due to the instability of the target compounds, all procedures were completed as rapidly as possible (i.e. ≤ 24 h) and purified material was stored as a dry solid under Ar atmosphere in the dark at ≤ 4 °C. After fermentation in the production medium for two days, the mycelia were filtered. The filtrate was first extracted with dichloromethane (3 × 250 mL/L of culture) and then with ethyl acetate (3 × 250 mL/L of culture). According to HPLC, the dichloromethane layer contained negligible amounts of the target compounds. The combined ethyl acetate extracts were washed with saturated brine (2/3 of equal volume), dried (Na_2SO_4), and evaporated *in vacuo* (≤ 25 °C) to remove the solvent. Purification was achieved by HPLC on a μBondapak radial compression reverse phase C_{18}, 40 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON). The flow rate was kept at 35 mL/min, and the gradient was raised from 30% acetonitrile in water and 0.1% TFA, to 50% over 12 min, kept at 50% for 5 min, raised from 50% to 70% over 6 min, from 70% to 100% over 2 min, and kept at 100% for 5 min. The UV detector was set at 360 nm. Retention times were 13 min for **41**, **41a**, **41b**, and **41c**, and 17 min for **42**, **42a**, **42b**, and **42c**. The latter appeared as two peaks which displayed identical spectroscopic and chromatographic characteristics including HPLC behaviour on reinjection (i.e. same two peaks), suggesting possible tautomeric equilibration.

***In vivo* incorporation studies of diketide 29a into 4a,5-dihydromonacolin L (11) using the *A. nidulans lovB+lovC* strain.** The *A. nidulans lovB+lovC* strain was fermented as described above. A solution of compound **29a** (22 mg) in absolute ethanol (2 mL), and a solution of the β-oxidation inhibitor **26** were used for the feedings. The injection of both solutions into the culture (1 L) started after the transfer from the YEPD to the AMM media. The addition of 0.12 mL each of both solutions was carried on every 4 hours for three days. 24 hours after the last addition of diketide **29a** and

inhibitor **26**, 4a,5-dihydromonacolin L (**11**) was purified using the procedure described above for its original isolation. According to the ^{13}C NMR of **11** obtained (10 mg), no incorporation of the diketide **29a** occurred.

Investigation of the effect of feeding sodium acetate or diethyl malonate to *A. nidulans lovB+lovC* cultures on the production of 4a,5-dihydromonacolin L (11). The *A. nidulans lovB+lovC* strain was fermented as described above. The feeding of sodium acetate and diethyl malonate separately into *A. nidulans lovB+lovC* cultures (1 L each) started after the transfer from the YEPD to the AMM media. The sodium acetate solution (6 × 5 mL of 10% aqueous) and the diethyl malonate (6 × 0.5 mL of neat liquid) were injected approximately every eight hours for two days. The cultures fed with diethyl malonate looked less pigmented than control cultures. Twenty-four hours after the last feeding, 4a,5-dihydromonacolin L (**11**) was purified using the procedure as described above for its original isolation. In the case where sodium acetate was fed, the production of 4a,5-dihydromonacolin L (**11**) (10 mg/L of cultures) appeared same as the control strain. On the other hand, diethyl malonate reduced the production of **11** below the limit of detection (ca. 0.05 mg) for the conditions used.

Fermentation of *Aspergillus terreus lovA* (P450I), *lovC* (dehydrogenase), *lovD* (esterase), and *lovF* (LDKS) mutants. Four *A. terreus* gene disruptant strains were provided by Professor C. R. Hutchinson (University of Wisconsin):[94] *A. terreus lovA* (P450I) (UAMH 9297), *A. terreus lovC* (dehydrogenase) (UAMH 9298 or WMH1734), *A. terreus lovD* (esterase) (UAMH 9295 or WMH1732), and *A. terreus lovF* (LDKS) (UAMH 9296 or WMH1731). They represent mutants in which the gene from the lovastatin cluster indicated by the name have been inactivated by insertion of foreign DNA. These strains were maintained on agar slants made from 5 g of Bacto-agar, 25 g of Pablum, and 250 mL of water. After inoculation, the fungi were incubated

at 28 °C for 8 days, after which they were stored at 4 °C. The *A. terreus* mutants were fermented and worked up using either the same method as for *A. nidulans* without PABA and cyclopentanone, the same method as for *A. terreus* XS-1P (see above), or the following. The required trace elements solution was prepared from: 1.6 g of $MnSO_4$, 2.0 g of $CoCl_2.6H_2O$, 3.4 g of $ZnSO_4.7H_2O$, and 5.0 g of $FeSO_4.7H_2O$, in 1 L of water. The fermentation medium contained: 0.5 mL of trace elements solution, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of NaCl, 1 g of KNO_3, 2 g of KH_2PO_4, 3 g of corn steep solids, and 20 g of lactose, diluted in 1 L of water at pH 6.6. The fermentation medium (500 mL in 2 L Erlenmeyer) was inoculated with one slant of the appropriate strain and incubated at 28 °C and 220 rpm for 6 days. The cultures (1 L) were vacuum filtered through two filter papers, and the filtrate was extracted with ethyl acetate (3 × 150 mL). The wet mycelia (30-45 g) were acidified to pH 3 (2N HCl), blended for 30 sec in water (100 mL), and for 1 min after ethyl acetate (200 mL) had been added. The combined organic layers were washed with brine, dried (Na_2SO_4), and the solvent was evaporated *in vacuo*. The residue was analyzed by HPLC with a μBondapak radial compression reverse phase column C_{18}, 25 × 100 mm, 10 μm, 125 Å (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min and a few different gradients with acetonitrile in water and 0.1% TFA were attempted. The UV detector was set at either 220, 240, or 360 nm. Neither mutant produced any lovastatin (**1**) under any of the fermentation methods used. The only new metabolite that could be detected in the *A. terreus lovD* and *lovF* mutants cultures by HPLC was monacolin J (**14**). When the *A. terreus lovC* mutant was fermented and worked up with the method described above for *A. nidulans*, the cultures showed production of yellow metabolites. A compound was purified in the same matter as for *A. nidulans lovB* and found to be compound **41** by IR, UV, ^{1}H NMR and ESMS (1.5 mg for 6 L of cultures). It was observed that the second pigment **42** was produced by the cultures as well (from the HPLC trace) although in too small amounts

for isolation. The extra gene in *A. nidulans lovB* was overexpressed, however this was not the case in *A. terreus lovC*, hence the smaller quantities.

Investigating the activity of the LovF protein (LDKS) in the *Aspergillus terreus lovC* (dehydrogenase) mutant: looking for the side chain polyketide (2*R*)-methylbutyric acid (15) and for the putative intermediates tiglic acid (50) and crotonic acid (51). *A. terreus lovC* was fermented as described above for *A. nidulans* but without PABA and cyclopentanone, and worked up as before. The column used for the detection of the diketides was a Novapak radial compression reverse phase C_{18}, 8 × 100 mm, 4 μm (Millipore Waters, Mississauga, ON). The flow rate was kept at 2 mL/min, and the gradient was raised from 0% to 10% acetonitrile in water and 0.1% TFA over 10 min, raised from 10% to 52% over 15 min, raised from 52% to 100% over 5 min, and kept at 100% for 5 min. The UV detector was set at 220 nm. Retention time was 15.6 min for (2*R*)-methylbutyric acid (**15**), 17.0 min for tiglic acid (**50**) and 38.9 min for crotonic acid (**51**).

Incorporation of monacolin J (14) into lovastatin (1) using the *A. terreus lovC* (dehydrogenase) mutant: investigating the activity of the LovF protein (LDKS). As shown above, no (2*R*)-methylbutyric acid (**15**), tiglic acid (**50**), nor crotonic acid (**51**) were detected in the *A. terreus lovC* cultures, suggesting that the diketide might remain loaded as a thioester on the enzyme. To test this hypothesis, the *A. terreus lovC* strain was fermented as described above, and starting 16 hours after the transfer, a solution of monacolin J (**14**) (0.1 mL of 30 mg in 1.2 mL of absolute ethanol) was fed to the growing cultures (1 L) every 6 hours for three days. The cultures were grown for an additional three days and then worked up as follows. The wet mycelia (45 g/L) were vacuum filtered, and the acidified filtrate (pH 2, 2N HCl) was extracted with ethyl acetate (3 × 350 mL). The combined organic layers were washed with brine and

dried (Na_2SO_4). After evaporation of the solvent, the residue was redissolved in toluene (60 mL) and heated under reflux with a soxhlet containing calcium hydride for one hour. The toluene was evaporated *in vacuo*, and the residue (0.28 g) was purified by HPLC on a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was kept at 40% acetonitrile in water and 0.1% TFA for 10 min, raised from 40% to 49% over 11 min, kept at 49% for 8 min, raised from 49% to 56% over 8 min, then raised from 56% to 95% over 2 min, and kept at 95% for 3 min. The UV detector was set at 220 nm. Retention time was 17.2 min for lovastatin (**1**) and 5.1 min for monacolin J (**14**). The presence of monacolin J (**14**) was also confirmed using the following gradient: kept at 25% acetonitrile in water and 0.1% TFA for 5 min, raised from 25% to 50% over 15 min, raised from 50% to 100% over 5 min, and kept at 100% for 3 min. The UV detector was set at 240 nm. The retention time of monacolin J (**14**) was 13.4 min. From the purification of about 1/3 of the culture extract, the yield of lovastatin (**1**) was estimated to 25 mg /L of cultures (70% from monacolin J), and about 5 mg/L of recovered monacolin J (**14**) (15%).

Incorporation of 6-desmethylmonacolin J (52) into compactin (5) using the *A. terreus lovC* (dehydrogenase) mutant: investigating the specificity of the transesterase (LovD protein). In order to investigate the specificity of the LovD protein (esterase), 6-desmethylmonacolin J (**52**) was fed to the *A. terreus lovC* cultures leading to the production of compactin (**5**) instead of lovastatin (**1**). The *A. terreus lovC* mutant was fermented as described above for *A. nidulans* but without PABA and cyclopentanone. Starting 16 hours after the media transfer, a solution of 6-desmethylmonacolin J (**52**) (0.1 mL of 25 mg in 1.0 mL of absolute ethanol) was fed to the growing cultures (1 L) every 6 hours for three days. The cultures were grown for three more days and then worked up as follows. The wet mycelia (63 g/L) were

vacuum filtered, and the acidified filtrate (pH 1, 2N HCl) was extracted with ethyl acetate (3 × 350 mL). The combined organic layers were washed with brine (500 mL) and dried (Na_2SO_4). After evaporation of the solvent, the residue (0.47 g) was redissolved in toluene (60 mL) and heated under reflux with a soxhlet containing calcium hydride for one hour. The toluene was evaporated *in vacuo*, and the residue (0.13 g) was purified by HPLC on a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was kept at 30% acetonitrile in water and 0.1% TFA for 5 min, raised from 30% to 49% over 16 min, raised from 49% to 95% over 8 min, and kept at 95% for 5 min. The UV detector was set at 238 nm. Retention time was 20.5 min for compactin (**5**). Further purification of **5** was achieved by filtration through a silica gel SEP-PAK® cartridge (Millipore Waters, Mississauga, ON) with 50% ethyl acetate in hexanes, and a Plus C_{18} SEP-PAK® cartridge using methanol. From the purification of about 1/3 of the culture extract, the yield of compactin (**5**) was estimated to be 11 mg/L of cultures (45% from 6-desmethylmonacolin J (**52**)).

Incorporation of 4a,5-dihydromonacolin L (11) into lovastatin (1) using the *A. terreus lovC* (dehydrogenase) mutant: confirmation of 11 as a biosynthetic precursor to lovastatin (1). The *A. terreus lovC* mutant is the strain in which the *lovC* gene has been disrupted (see above) and therefore produces yellow pigments, but no lovastatin (**1**). The *A. terreus lovC* mutant is believed to still contain all the machinery to make lovastatin except for the three enoyl reductions believed to be carried on by the LovC protein during the assembly of 4a,5-dihydromonacolin L (**11**). To test this hypothesis, 4a,5-dihydromonacolin L (**11**) was fed to the *A. terreus lovC* mutant and the production of lovastatin (**1**) was verified. The *A. terreus lovC* strain was fermented as described above for *A. nidulans* but without PABA and cyclopentanone. Starting after the transfer, a solution of 4a,5-dihydromonacolin L (**11**) (0.1 mL of 12 mg in 2.0 mL of

absolute ethanol) was fed to the growing cultures (1 L) every 4 hours for three days. The cultures were grown for an additional three days and then worked up as follows. The wet mycelia (36 g/L) were vacuum filtered, and the acidified filtrate (pH 2, 2N HCl) was extracted with ethyl acetate (3 × 300 mL), and the combined organic layers were washed with brine (600 mL) and dried (Na_2SO_4). After evaporation of the solvent, the residue (165 mg) was redissolved in toluene (60 mL) and heated under reflux with a soxhlet containing calcium hydride for one hour. The toluene was concentrated *in vacuo*, and the residue (71 mg) was purified by HPLC on a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was kept at 40% acetonitrile in water and 0.1% TFA for 10 min, raised from 40% to 49% over 11 min, raised from 49% to 56% over 4 min, from 56% to 100% over 2 min, and kept at 100% for 3 min. The UV detector was set at 238 nm. Lovastatin (**1**) had a retention time of 16.9 min. The yield of lovastatin (**1**) was 6 mg/ L of cultures (40% from 4a,5-dihydromonacolin L (**11**)).

Incorporation studies of ML-236C (25) into compactin (5) using the *A. terreus lovC* (dehydrogenase) mutant: investigating the specificity of the post-PKS enzymes. The *A. terreus lovC* mutant is believed to have all the machinery to make lovastatin (**1**) except for the three enoyl reductions believed to be carried on by the LovC protein. In order to investigate the specificity of the post-PKS enzymes ML-236C (**25**) was fed to verify the production of compactin (**5**). The *A. terreus lovC* strain was fermented as described above for *A. nidulans* but without PABA and cyclopentanone. Starting after the transfer, a solution of ML-236C (**25**) (0.1 mL of 9 mg in 2.0 mL of absolute ethanol) was fed to the growing cultures (1 L) every 4 hours for three days. The cultures were grown for an additional three days and then worked up as follows. The wet mycelia (42 g/L) were vacuum filtered, and the acidified filtrate

(pH 2, 2N HCl) was extracted with ethyl acetate (3 × 300 mL), and the combined organic layers were washed with brine (600 mL) and dried (Na_2SO_4). After evaporation of the solvent, the residue (146 mg) was redissolved in toluene (60 mL) and heated under reflux with a soxhlet containing calcium hydride for one hour. The toluene was evaporated *in vacuo*, and the residue (49 mg) was purified by HPLC on a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON). The flow rate was 15 mL/min, and the gradient was kept at 30% acetonitrile in water and 0.1% TFA for 5 min, raised from 30% to 49% over 16 min, raised from 49% to 95% over 8 min, and kept at 95% for 5 min. The UV detector was set at 238 nm. The retention time of compactin (**5**) was 20.5 min. No peaks were observed around 20 min for the culture extract, and ML-236C (**25**) could not be detected.

4. Cell Free Extracts (CFEs) and LNKS Purification

Studies for preparation of cell free extracts from the *Aspergillus terreus lovE* strain. The *A. terreus lovE* strain was fermented as described above. After two days of incubation in production media, preparation of CFEs was attempted using the method of Watanabe and Craig[111] with some modifications. The mature cultures (750 mL) were vacuum filtered through 4 layers of cheesecloth and rinsed with water (1L). All subsequent steps were done at 4 °C. The wet mycelia (ca. 100 g/L) were flash frozen in liquid nitrogen, then blended with dry ice (4 × 30 sec). The resulting broken cells were suspended in extraction buffer (500 mL or 600 mL of 50 mM potassium phosphate at pH 7.5, 50% glycerol, 1 mM EDTA, and 2 mM β-mercaptoethanol, either with or without 0.1 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride predissolved in a minimum of acetone) and shaken at 75 rpm for two hours. The cell

debris was removed by centrifugation at 4 °C and 12000 × *g* for 20 min twice, and the supernatant was filtered through four layers of cheesecloth. Using the Amicon filtration apparatus (300 mL) with one of 10000 or 100000 MW cut off membranes (YP10 or YM100), the supernatant was dialyzed against 4 L of buffer (extraction buffer or the following buffer: 100 mM potassium phosphate at pH 7.5, 30% glycerol, 1 mM EDTA, 2 mM DTT) at 45 or 50 psi, until the volume was reduced to about 250 mL. The solution present in the Amicon cell at this stage was the CFE. The enzyme assay was performed as described for *A. terreus* XS-1P below, but no activity was found.

Studies for preparation of cell free extracts from the *Aspergillus terreus* XS-1P strain. The *A. terreus* XS-1P strain was fermented in Seed Medium (2 × 500 mL) for two days as described above for lovastatin (**1**) quantification. Preparation of CFEs was attempted using the method of Watanabe and Craig[111] with some modifications. The mature cultures (1 L, before transfer into Fermentation Medium) were vacuum filtered through 4 layers of cheesecloth. All the subsequent steps were done at 4 °C. The wet mycelia (ca. 40 g/L) were flash frozen in liquid nitrogen, the blended with dry ice (4 × 30 sec). The cells were also broken at 4 °C using a bead beater (4 × 30 sec with 30 g of 3 mm beads, 3 × 30 sec with 50 g of beads, or 6 × 30 sec with 100 g of beads) or a sonicator (3 × 30 sec, or 6 × 30 sec). The resulting broken cells were suspended in extraction buffer (300 mL of 50 mM potassium phosphate at pH 6.5, 30% glycerol, 1 mM EDTA, 2 mM β-mercaptoethanol, 0.1 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride predissolved in a minimum of acetone) and shaken at 75 rpm for two hours. The cell debris was removed by centrifugation at 4 °C and 12000 × *g* for 30 min, and the supernatant was filtered through four layers of cheesecloth. Using the Amicon 2000 filtration apparatus with one of 10000 or 100000 MW cut off membranes (YP10 or YM100), the supernatant was dialyzed against 4 L of buffer (extraction buffer or the following buffer: 100 mM potassium phosphate at pH 7.5, 30%

glycerol, 1 mM EDTA, 2 mM DTT) at 45 or 50 psi, until the volume was reduced to about 250 mL. The solution present in the Amicon cell at this stage was the CFE. The enzyme assay was performed as follows. For each method attempted, the assay included three controls and two reaction mixtures. Control 1 contained only the CFE (10 mL). Control 2 was a mixture of CFE (10 mL) and cofactor solution (100 µL of a solution containing 5 mg of NADPH, 5 mg of FAD, and 5 mg of SAM, in 5 mL of water). Control 3 was prepared from: CFE (10 mL), acetyl CoA (1 mg), and malonyl CoA (1 mg). Reaction 1 was a mixture of CFE (10 mL), cofactor solution (100 µL), acetyl CoA (1 mg), malonyl CoA (5 mg), and [2-^{14}C]-malonyl CoA (20-30 µL of an acidic aqueous solution, 0.20-0.30 µCi). Reaction 2 contained: CFE (10 mL), cofactor solution (100 µL), acetyl CoA (1 mg), malonyl CoA (5 mg), and [methyl-^{3}H]-*S*-adenosyl-*L*-methionine (1 µL of an aqueous solution, 0.5 µCi). The five solutions were left on an orbital shaker at r.t. and 25 rpm overnight, for one or for two days. The solutions were extracted with ethyl acetate (3 × 20 mL each), and the combined organic layers were individually washed with brine (50 mL) and dried (Na_2SO_4). After evaporation of the solvent *in vacuo*, the distribution of the radioactivity in reaction 1 and reaction 2 was determined using scintillation counter for each fraction collected by HPLC. Analytical HPLC was performed using a Novapak radial compression reverse phase C_{18}, 8 × 100 mm, 4 µm column (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 20% acetonitrile in water and 0.1% TFA for 10 min, then went from 20% to 21% over 4.5 min, from 21% to 23.5% over 1.5 min, from 23.5% to 28% over 1.5 min, from 28% to 37.5% over 1 min, from 37.5% to 50% over 0.5 min, from 50% to 90% over 1 min, from 90% to 100% over 1 min, and kept at 100% for 5 min. The UV detector was set at 225 or 360 nm. Preparative HPLC was performed using a µBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 µm, 125 Å column (Millipore Waters, Mississauga, ON) ran at 15 mL/min. The gradient started at 40% acetonitrile in water and 0.1% TFA, then went from 40% to

50% over 10 min, from 21% to 23.5% over 1.5 min, from 23.5% to 28% over 1.5 min, from 28% to 37.5% over 1 min, from 50% to 70% over 5 min, from 70% to 100% over 3 min, and kept at 100% for 5 min. The UV detector was set at 225 or 360 nm. Fractions were collected at two minute intervals and the radioactivity was determined by scintillation counting. None of the fractions collected showed significant radioactivity for both ^{14}C and ^{3}H. Most of the ^{14}C radioactivity was found to have remained in the aqueous layer (95%). The [2-^{14}C]-malonyl CoA was tested for potential decomposition using HPLC of labeled and non-labeled malonyl CoA followed by counting of ^{14}C. [2-^{14}C]-Malonyl CoA was found to be 79% pure.

Preparation of cell free extracts from *A. nidulans* strains. A modification of the method of C. D. Reeves was used.[93] The mature culture (1 L) of *A. nidulans* (either the *lovB* or the *lovB+lovC* strains) was gravity filtered through a Miracloth and the mycelia were washed with Milli-Q water (3.5 L). The wet mycelia (ca. 15 g) were frozen in a dry ice/acetone bath and freeze-dried overnight. All the following steps were done at 4 °C. The dried mycelia (ca. 3 g) were pulverized in extraction buffer (25 mL of: 20 mM Tris-HCl pH 8.0, 10% glycerol, 1 M NaCl, 5 mM sodium ascorbate, 1 mM EDTA, 1 mM DTT, 3.8 µg/mL of leupeptin, 17.7 µg/mL of chymostatin, 2 µg/mL of pepstatin, 42 µg/mL of trypsin inhibitor, 0.2 mM of phenylmethylsulfonylfluoride, and 2.2% polyvinyl polypyrrolidone) using a mortar and pestle for at least 10 min. The broken cell suspension was diluted with more extraction buffer (175 mL) and left to extract on an orbital shaker at 75 rpm for one hour. The cell debris was separated by centrifugation at 10000 × *g* for 30 min. The supernatant was gravity filtered through 4 layers of cheesecloth, affording the CFE.

LNKS (LovB protein) enzyme assay. The cofactor solution contained 25 mg of SAM, 25 mg of NADPH, and 25 mg of FAD, in 25 mL of Milli-Q water. The blank

assay was prepared by adding the cofactor solution (20 μL) to the fresh cell free extract (1 mL). The enzyme assay solution was prepared by mixing cell free extract (1 mL), cofactor solution (20 μL), acetyl CoA (1 mg), and malonyl CoA (3 mg). The enzyme activity was measured by following the production of the pigments **41** and **42** at 365 nm and 26 °C for 10 hours. Although the *A. nidulans lovB+lovC* strain produces mainly 4a,5-dihydromonacolin L (**11**), the pigments **41** and **42** are also produced by LNKS, and its activity can therefore be monitored by UV. The maximum production (maximum absorbance) was achieved within a few hours for the *A. nidulans lovB* strain.

***A. nidulans lovB+lovC* cell free production of 4a,5-dihydromonacolin L (11).** To the cell free extract of the *A. nidulans lovB+lovC* strain (40 mL) prepared as above was added the cofactor solution (0.5 mL of 2.5% (w/v) of each NADPH, FAD, SAM), acetyl CoA (5 mg) and malonyl CoA (20 mg). The solution was left on an orbital shaker at r.t. and 50 rpm overnight. The reaction mixture was quenched by acidification to pH 2 (2N HCl) and extracted with ethyl acetate (4 × 40 mL). The combined organic layers were washed with brine (150 mL), dried (Na_2SO_4), and concentrated *in vacuo*. In order to ensure that all the 4a,5-dihydromonacolin L (**11**) was lactonized, toluene was added (25 mL) to the residue and the solution was heated under reflux with a sohxlet containing calcium hydride for 45 min. The toluene was removed *in vacuo* and the 4a,5-dihydromonacolin L (**11**) was purified by HPLC using the same conditions as for its original isolation. Compound **11** was found to be produced by the *A. nidulans lovB+lovC* CFE (<0.1 mg).

***A. nidulans lovB+lovC* cell free incorporation studies with propionyl CoA and butyryl CoA.** The cell free extract of the *A. nidulans lovB+lovC* strain was prepared and tested as described above. Both incorporation assay mixtures contained the CFE (45 mL), the cofactor solution (0.5 mL of a solution of 5 mg of NADPH, 5 mg

of FAD, and 5 mg of SAM, in 5 mL of water), malonyl CoA (55 mg), and propionyl CoA (20 mg, 0.024 mmol) or butyryl CoA (25 mg, 0.030 mmol). The reaction mixtures were left on an orbital shaker at r.t. and 50 rpm overnight. The two assays were quenched and worked up separately. The acidified mixtures (pH 1, 2N HCl) were extracted with ethyl acetate (3 × 100 mL each). The organic layers were washed with brine (300 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The residues (43 mg from the propionyl CoA assay, and 64 mg for butyryl CoA) were redissolved in toluene (25 mL each) and heated under reflux for one hour with a soxhlet containing calcium hydride. After evaporation of the toluene, the residues (24 mg, and 30 mg respectively) were purified by HPLC. Preparative HPLC was performed using a μBondapak radial compression reverse phase C_{18}, 25 × 100 mm, 10 μm, 125 Å column (Millipore Waters, Mississauga, ON) ran at 15 mL/min. The gradient started at 30% acetonitrile in water and 0.1% TFA, went from 30% to 40% over 10 min, from 40% to 49% over 11 min, kept at 49% for 6 min, raised from 49% to 100% over 3 min, and kept at 100% for 3 min. The UV detector was set at 200 nm. No new peaks were detected when propionyl CoA was used. A new peak was observed for the butyryl CoA assay and was believed to be recovered starting material (same retention time).

***In vitro* incorporation studies of diketide 29a into 4a,5-dihydromonacolin L (11) using *A. nidulans lovB+lovC* CFE.** To the cell free extract of the *A. nidulans lovB+lovC* strain (35 mL) prepared and tested as above was added the cofactor solution (0.5 mL of 2.5% (w/v) of each NADPH, FAD, and SAM), malonyl CoA (40 mg), and a solution of diketide **29a** (10 mg, 0.04 mmol) in absolute ethanol (ca. 100 μL). The solution was left on an orbital shaker at r.t. and 50 rpm overnight. The reaction mixture was quenched by acidification to pH 2 (2N HCl), extracted with ethyl acetate (4 × 70 mL), and the combined organic layers were washed with brine (250 mL), dried (Na_2SO_4), and evaporated *in vacuo*. In order to ensure that all the 4a,5-

dihydromonacolin L (**11**) was lactonized, toluene was added (25 mL) to the residue and the solution was heated under reflux with a sohxlet containing calcium hydride for 45 min. The toluene was removed *in vacuo* and the 4a,5-dihydromonacolin L (**11**) as purified by HPLC using the same conditions as for its original isolation. According to MS no incorporation of the intact diketide **29a** occurred, very likely because of rapid degradation (scrambling of the ^{13}C observed in MS of crude).

Incorporation studies of [2,11-$^{13}C_2$]-(2*E*,8*E*,10*E*)-(*R*)-6-methyldodecatri-2,8,10-enoic acid, *N*-acetylcysteamine thioester (16a) into 4a,5-dihydromonacolin L (11) using *A. nidulans lovB+lovC* CFE. To the cell free extract of the *A. nidulans lovB+lovC* strain (100 mL) prepared as above was added the cofactor solution (1.0 mL of 2.5% (w/v) of each NADPH, FAD, and SAM), malonyl CoA (100 mg), and a solution of triene **16a** (21 mg, 0.068 mmol) in absolute ethanol (0.44 mL). The solution was left on an orbital shaker at r.t. and 50 rpm overnight. The reaction mixture was quenched by acidification to pH 2 (2N HCl), extracted with ethyl acetate (4 × 150 mL), and the combined organic layers were washed with brine (500 mL), dried (Na_2SO_4), and evaporated *in vacuo*. In order to ensure that all the 4a,5-dihydromonacolin L (**11**) was lactonized, toluene was added (25 mL) to the residue and the solution was heated under reflux with a sohxlet containing calcium hydride for 60 min. The toluene was removed *in vacuo* and the 4a,5-dihydromonacolin L (**11**) was purified by HPLC using the same conditions as for its original isolation. According to MS no intact incorporation of the compound **16a** occurred very likely because of rapid degradation (scrambling of the ^{13}C observed in the MS of the crude).

Purification of LNKS (LovB protein). A modification of the method of Reeves and coworkers was used.[93] The *A. nidulans lovB* cell free extract (150 mL prepared as above) was subjected to dialysis using the Amicon 200 apparatus (100000

MW cut-off YM100 membrane) until the solution volume was concentrated by about 7 times (~20 mL). The concentrated cell free extract was loaded onto a Sephadex G-150 (1 L) or G-200 column (600 mL) equilibrated at 4 °C in elution buffer consisting of: 20 mM Tris-HCl, 10% glycerol, 0.1 M NaCl, 1 mM sodium ascorbate, 1 mM EDTA, 1 mM DTT, and 0.1% Tween 80. Fractions of 8.0 mL were collected at 0.8 mL/min. The protein concentration was monitored at 280 nm. The fractions were pooled and the protein was precipitated by adding ammonium sulfate to 60% saturation and centrifuging twice at 10000 × *g* for 30 min. The collected proteins were resuspended in pellet buffer (20 mM Tris pH 8.0, 1 mM DTT, and 1 mM sodium ascorbate). Aliquots were used for SDS-PAGE and enzyme assay of the ~300 kDa protein. The Bio-Rad 5% SDS-PAGE Ready Gels were ran at 150 V for 80 min and stained with Coomassie Blue. Bio-Rad 12% SDS-PAGE Ready Gels were also ran, but staining with Coomassie Blue revealed only one band near the well. As shown by SDS-PAGE, the first pooled protein sample of the size exclusion column contained a protein of about 600 kDa. The second pooled protein sample consisted of mainly the desired protein based on its predicted molecular weight of 335 kDa. A minor protein (~ 260 kDa) observed in the latter is believed to arise from decomposition of the target protein during precipitation since it is not observed in the crude extract. The protein pellet of fraction two was a beige solid.

5. Enzymatic Tests for Compounds 16, 59, 60, 78, 82, 92, 93, and 94.

LNKS enzyme-catalyzed Diels-Alder reaction of triene 16 affording (1*S*,2*S*,4a*R*,6*R*,8a*S*)-1,2-4a,5,6,7,8a-octahydro-2,6-dimethylnaphthalene-1-carboxylic acid, *N*-acetylcysteamine thioester (56). The triene **16** was shown to undergo a stereoselective Diels-Alder reaction catalyzed by LNKS leading to compound **56** (see the compounds section under **56**).

LNKS reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (59). The stability of the triene **59** in presence of water was tested by ^{1}H NMR in D_2O:DMSO-d_6 2:1 (minimum DMSO required to dissolve enough sample for detection; milky solution). The ^{1}H NMR was unchanged after one week. The enzymatic reaction was performed as follows. The protein pellet obtained from the purification of LNKS (~ 5 mg) was redissolved in pellet buffer (5 mL, see cell free extracts/enzyme purification section). This solution (1 mL) was diluted with more buffer (15 mL), water (15 mL) and ethanol (5 mL) to help dissolve the added hydrophobic triene **59** (55 mg, 0.14 mmol). The reaction mixture was left on an orbital shaker at r.t. and 75 rpm for two days. More ethanol (6 mL) was added to help solubility and the mixture was left on the shaker for one more day. The biphasic milky solution was extracted with ethyl acetate (3 × 75 mL), and the combined organic layers were washed with brine (200 mL), dried (Na_2SO_4), and evaporated *in vacuo*, affording pure recovered material **59** (54 mg, 98%).

Horse liver alcohol dehydrogenase reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (59): first method. Horse liver alcohol dehydrogenase (HLADH; alcohol:NAD^+ oxidoreductase; EC 1.1.1.1) had an activity of 1.5 units/mg solid. Unit definition: One unit will convert 1 μmol of ethanol per minute at pH 8.8 and 25 °C. The enzyme was assayed by monitoring the transformation of NAD to NADH in presence of ethanol at 340 nm, and the protein concentration was estimated based on absorbance at 280 nm.[130] The enzyme HLADH and cofactor NAD were immobilized on glass beads (106 microns and finer, Sigma) and the reaction was performed using a modification of the method described by Grunwald *et al.*[129] The HLADH (40 mg, 60 U), glass beads (2 g), and a solution of NAD (1 mL of 2 mg in 500 mL) in buffer (20 mM Tris-HCl pH 7.0) were spread on a watch glass and

occasionally mixed until freely flowing. The blank reaction contained freshly distilled isopropyl ether saturated in buffer (28 mL) and immobilized enzyme/cofactor (0.29 g). The enzymatic reaction was prepared by mixing the substrate **59** (50 mg, 0.13 mmol), isopropyl ether saturated in buffer (2.6 mL), isobutyraldehyde (0.024 mL), buffer (5.2 μL) and immobilized enzyme/cofactor (0.28 g, 0.4 μmol NAD, 8.4 U of HLADH). The heterogeneous solution was left on an orbital shaker at r.t. and 250 rpm for one week, at which time most of the isopropyl ether had evaporated, so more was added (4 mL) and the mixture was shaken for another 6 days. The reaction was followed by UV and TLC and showed no reaction. The enzyme/cofactor were filtered and washed with isopropyl ether. The filtrate was evaporated *in vacuo*, affording pure recovered starting material **59** (36 mg, 75%).

Horse liver alcohol dehydrogenase reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (59): second method. The enzymatic reaction was prepared by adding the substrate **59** (15 mg, 0.04 mmol) to a solution containing HLADH (10 mg, 15 U, see above), NAD (31 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). The blank reaction was prepared in the same way but no substrate was added. The mixtures were left at r.t. without shaking for 9 days. The reactions were quenched by extracting with diethyl ether (4 × 20 mL). The emulsions were filtered through glass wool. The combined organic layers were washed with brine (80 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residue was transferred into a Nalgene container. Methanol (0.7 mL) and 48% HF (0.1 mL) were added in order to cleave the diphenylsilyl tether. After 8 hours at r.t., the mixture was neutralized (sodium hydrogen carbonate) and diluted with water (10 mL). After extraction with diethyl ether (3 × 15 mL), drying (Na_2SO_4), and evaporation of the solvent, the residue (1 mg) did not appear to contain compound **68** by TLC and HPLC. Analytical separation by HPLC was

achieved using a Novapak radial compression reverse phase C_{18}, 8 × 100 mm, 4 μm column (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 30% of 80% acetonitrile in water for 5 min, then went from 30% to 95% over 30 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. The retention time was 28 min for the standard compound **68**.

Baker's yeast alcohol dehydrogenase reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (59): first method. Baker's yeast alcohol dehydrogenase (BYADH; alcohol:NAD^+ oxidoreductase; EC 1.1.1.1) had an activity of 290 units/mg. Unit definition: One unit will convert 1 μmol of ethanol per minute at pH 8.8 and 25°C. The enzyme was assayed by monitoring the transformation of NAD to NADH in presence of ethanol at 340 nm and the protein concentration at 280 nm.[130] The enzyme BYADH and cofactor NAD were immobilized on glass beads in the same way as for HLADH. The blank reaction was a mixture of freshly distilled isopropyl ether (7 mL) saturated in buffer (20 mM Tris-HCl, pH 7.0), buffer (0.05 mL), and immobilized enzyme/cofactor (3.0 g). The enzymatic reaction was prepared by adding the substrate **59** (32 mg, 0.082 mmol) to isopropyl ether (7 mL), buffer (0.05 mL), and enzyme/cofactor (3.0 g, 291 U). The mixtures were left on an orbital shaker at r.t. and 250 rpm for 10 days. The reaction was followed by TLC and showed no change. The beads were filtered and the evaporated filtrate contained pure recovered compound **59** (32 mg, quant.).

Baker's yeast alcohol dehydrogenase reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E*,*E*)-2,4-hexadien-1-oxy] diphenylsilane (59): second method. The enzymatic reaction was prepared by adding the substrate **59** (15 mg, 0.04 mmol) to a solution containing: BYADH (1.5 mg, 290 U, see above), NAD (32 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). The blank

reaction was prepared in the same way but no substrate was added. The mixtures were left at r.t. without shaking for 9 days. The reactions were quenched by extraction in diethyl ether (4 × 20 mL), and the resulting emulsions were filtered through glass wool. The combined organic layers were washed with brine (80 mL), dried ($MgSO_4$), and after evaporation of the solvent, the residue was transferred into a Nalgene container. Methanol (0.7 mL) and 48% HF (0.1 mL) were added in order to cleave the diphenylsilyl tether. After 8 hours at r.t., the mixture was neutralized (sodium hydrogen carbonate), and diluted with water (10 mL). Extraction with diethyl ether, drying (Na_2SO_4), and evaporation of the solvent, afforded the residue (5 mg) which did not appear to contain compound **68** as determined by TLC and HPLC. Analytical separation by HPLC was achieved by using a Novapak radial compression reverse phase C_{18}, 8 × 100 mm, 4 µm column (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 30% of 80% acetonitrile in water for 5 min, then went from 30% to 95% over 30 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. The retention time was 28 min for the standard compound **68**.

Subtilisin Carlsberg transesterification reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (59) and methanol. Subtilisin Carlsberg from *Bacillus licheniformis* (protease type VIII:bacteria; subtilopeptidase A; EC 3.4.21.62) was purchased with an activity of 7-15 units/mg. The enzyme was assayed using the "Folin and Ciocalteu's Phenol Reagent for Protein Determination" (Sigma kit, procedure no. 690) and found to have an activity of 0.4 units/mg. Unit definition: One unit will hydrolyse casein to produce the color equivalent to 1.0 µmol (181 µg) of tyrosine per minute at 37 °C and pH 7.5. The enzymatic reaction was performed using a modification of the method described by Zaks and Klibanov.[125] Subtilisin Carlsberg (75.8 mg) was dissolved in buffer (15 mL of 20 mM phosphate buffer at pH 7.8) and frozen in a dry ice/acetone bath. The solution

was lyophilized for three days, affording the pH adjusted enzyme (total 118 mg, 64% enzyme). The enzyme assay blank was a mixture of freshly distilled *n*-octane (0.5 mL), dry methanol (0.33 mL, 5 mmol), and enzyme (10 mg of lyophilized enzyme, 2.6 U). The enzyme assay, run in parallel, was a solution of casein (50 mg) and enzyme (10 mg, 2.6 U) in *n*-octane (0.5 mL). The substrate blank contained *n*-octane (0.5 mL), methanol (0.33 mL, 5 mmol), and compound **59** (20 mg, 0.05 mmol). The enzymatic reaction was prepared by adding the substrate **59** (20 mg, 0.05 mmol) to a solution of enzyme (10 mg, 2.6 U) in *n*-octane (0.5 mL). The four mixtures were left on an orbital shaker at r.t. and 250 rpm for two weeks. The "Folin and Ciocalteus Reagent for Protein Determination" was used with the enzyme assay blank and reaction solutions to ensure that the enzyme was active. The enzymatic transesterification reaction and the blank were filtered through a sintered-glass funnel. The filter cake collected was washed with chloroform, and the filtrate was evaporated *in vacuo*. Unchanged substrate **59** was recovered for both solutions, along with some untethered substrate.

Carbonic anhydrase reaction with [3-carbomethoxy-(*E*)-2-propen-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (59). Carbonic anhydrase from bovine erythrocytes (carbonate dehydratase; carbonate hydro-lyase; EC 4.2.1.1) was purchased with an activity of 5800 WA units/mg. Wilburg-Anderson (WA) unit definition: One unit will cause the pH of 0.02 M Trizma buffer to drop from 8.3 to 6.3 per minute at 0 °C.[127] The enzyme was immobilized on glass beads using a modification of the procedure used for HLADH and BYADH. Glass beads (1.0 g, 106 microns and smaller, Sigma) were washed with buffer (50 mM Tris-HCl pH 7.8) and added to a solution of carbonic anhydrase (10.7 mg, 56632 WA units) in buffer (1 mL). The slurry was gently spread on a watch glass and allowed to dry. The blank reaction contained immobilized enzyme (0.25 g, 14000 WA units), isopropyl ether saturated in buffer (3 mL), and buffer (5.6 μL). The enzymatic reaction was prepared by adding the substrate

59 (53.7 mg, 0.136 mmol) to a mixture of immobilized enzyme (0.25 g, 14000 WA units), isopropyl ether saturated in buffer (3 mL), and buffer (5.6 μL). Both mixtures were left on an orbital shaker under Ar at r.t. and 250 rpm for one week. The reaction progress was followed by UV at 240 nm. The immobilized enzyme was filtered and washed with isopropyl ether. The filtrates were dried ($MgSO_4$) and evaporated *in vacuo*. Starting material **59** (50 mg, 93%) was recovered unchanged from the enzymatic reaction.

LNKS reaction with *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60). The stability of the triene **60** in presence of water was tested by ^1H NMR in D_2O:DMSO-d_6 2:1 (minimum DMSO required to dissolve enough sample for detection; milky solution). The ^1H NMR was unchanged after one week. The protein pellet obtained from the purification of LNKS (~ 5 mg) was redissolved in pellet buffer (5 mL, see the cell free extracs/enzyme purification section). This solution (1 mL) was diluted with more buffer (15 mL), water (15 mL), and ethanol (5 mL) to help dissolve the added hydrophobic triene **60** (65 mg, 0.18 mmol). The reaction mixture was left on an orbital shaker at r.t. and 75 rpm for two days. More ethanol (6 mL) was added to help solubility, and the mixture was left on the shaker for a further 24 hours. The biphasic milky solution was extracted with ethyl acetate (3 × 60 mL), and the combined organic layers were washed with brine (150 mL), dried (Na_2SO_4), and evaporated *in vacuo*, to afford pure recovered **60** (64 mg, 98%).

Horse liver alcohol dehydrogenase reaction with *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60): first method. Compound **60** was tested with HLADH as described above for compound **59**. Therefore, the enzymatic reaction was prepared by mixing substrate **60** (52 mg, 0.15

mmol), isopropyl ether saturated in buffer (2.8 mL), isobutyraldehyde (0.026 mL), buffer (5.6 μL), and immobilized enzyme/cofactor (0.26 g, 0.4 μmol NAD, 7.8 U of HLADH). The reaction was followed by UV and TLC and showed no reaction. Pure recovered starting material **60** (38 mg, 75%) was obtained after work up as above.

Horse liver alcohol dehydrogenase reaction with *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60): second method. Compound **60** was tested with HLADH as described above for compound **59**. Thus, the enzymatic reaction was prepared by adding substrate **60** (14 mg, 0.04 mmol) to a solution containing HLADH (10 mg, 15 U, see above), NAD (32 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). After work up, the residue did not appear to contain the expected Diels-Alder adducts **67** as shown by TLC and HPLC. Analytical separation by HPLC was achieved using a Resolve radial compression reverse phase C_{18}, 8 × 100 mm, 4 μm column (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 5% acetonitrile in water for 5 min, then went from 5% to 60% over 20 min, from 60% to 95% over 5 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. The retention times were 29.8 and 30.1 min for the standard Diels-Alder adducts **67**.

Baker's yeast alcohol dehydrogenase reaction with *cis*-(*E*,*E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60): first method. Compound **60** was tested with BYADH as described above for compound **59**. Therefore, the enzymatic reaction was prepared by adding the substrate **60** (29 mg, 0.082 mmol) to isopropyl ether (7 mL), buffer (0.05 mL), and enzyme/cofactor (3.0 g, 291 U). The reaction was followed by TLC and showed no reaction. Work up as above afforded pure recovered material **60** (29 mg, quant.).

Baker's yeast alcohol dehydrogenase reaction with *cis*-(*E,E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60): second method. Compound **60** was tested with BYADH as described above for compound **59**. Thus, the enzymatic reaction was prepared by adding substrate **60** (14 mg, 0.04 mmol) to a solution containing: BYADH (1.5 mg, 290 U, see above), NAD (33 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). After work up, the residue did not appear to contain the expected Diels-Alder adducts **67** as shown by TLC and HPLC. Analytical separation by HPLC was achieved using a Resolve radial compression reverse phase C_{18}, 8 × 100 mm, 4 μm column (Millipore Waters, Mississauga, ON) ran at 2 mL/min. The gradient started at 5% acetonitrile in water for 5 min, then went from 5% to 60% over 20 min, from 60% to 95% over 5 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. The retention times were 29.8 and 30.1 min for the standard Diels-Alder adducts **67**.

Subtilisin Carlsberg transesterification reaction with *cis*-(*E,E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60) and methanol. Compound **60** was tested with subtilisin Carlsberg as described above for compound **59**. Therefore, the enzymatic reaction was prepared by addition of the substrate **60** (18 mg, 0.05 mmol) to a solution of lyophilized enzyme (10 mg, 2.6 U) in *n*-octane (0.5 mL). Unchanged substrate **60** was recovered for both the blank and the enzymatic solutions.

Carbonic anhydrase reaction with *cis*-(*E,E*)-2,4-hexadien-1-yl (*E*)-3-carbomethoxy-2-propen-1-yl cyclohexane-1,2-dicarboxylate (60). Compound **60** was tested with carbonic anhydrase as described above for compound **59**. Thus, the enzymatic reaction was prepared by adding substrate **60** (53.7 mg, 0.136 mmol) to a mixture of immobilized enzyme (0.25 g, 14000 WA units), isopropyl ether saturated in

buffer (3 mL), and buffer (5.6 µL). The reaction progress was followed by UV at 240 nm. Starting material **60** (46 mg, 96%) was recovered unchanged from both the blank and the enzymatic reaction.

Horse liver alcohol dehydrogenase reaction with [4-hydroxy-(*E*)-2-buten-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (78): first method. Compound **78** was tested with HLADH as described above for compound **59**. Therefore, the enzymatic reaction was prepared by mixing the substrate **78** (50 mg, 0.14 mmol), isopropyl ether saturated in buffer (2.8 mL), isobutyraldehyde (0.025 mL), buffer (5.6 µL) and immobilized enzyme/cofactor (0.38 g, 0.5 µmol NAD, 11.4 U of HLADH). The reaction was followed by UV and TLC, and showed no reaction. Work up as before afforded pure recovered starting material **78** (42 mg, 85%).

Horse liver alcohol dehydrogenase reaction with [4-hydroxy-(*E*)-2-buten-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (78): second method. Compound **78** was tested with HLADH as described above for compound **59**. Thus, the enzymatic reaction was prepared by adding the substrate **78** (15 mg, 0.04 mmol) to a solution containing HLADH (10 mg, 15 U, see above), NAD (31 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). After work up as before, the residue was analysed by TLC and separated by HPLC. Preparative HPLC was achieved using a µBondapak radial compression reverse phase C_8, 25 × 100 mm, 300 Å, 15-20 µm column (Millipore Waters, Mississauga, ON) ran at 10 mL/min. The gradient started at 5% acetonitrile in water for 5 min, then went from 5% to 60% over 20 min, from 60% to 95 % over 5 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. Of the ten peaks collected, none displayed NMR data consistent with the expected Diels-Alder adduct structure.

Baker's yeast alcohol dehydrogenase reaction with [4-hydroxy-(*E*)-2-buten-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (78): first method. Compound **78** was tested with BYADH as described above for compound **59**. Therefore, the enzymatic reaction was prepared by adding the substrate **78** (30 mg, 0.082 mmol) to isopropyl ether (7 mL), buffer (0.05 mL), and enzyme/cofactor (3.0 g, 291 U). The reaction was followed by TLC and showed no reaction. Pure recovered material **78** (30 mg, quant.) was obtained after work up as above.

Baker's yeast alcohol dehydrogenase reaction with [4-hydroxy-(*E*)-2-buten-1-oxy]-[(*E,E*)-2,4-hexadien-1-oxy] diphenylsilane (78): second method. Compound **78** was tested with BYADH as described above for compound **59**. Thus, the enzymatic reaction was prepared by adding the substrate **78** (15 mg, 0.04 mmol) to a solution containing: BYADH (1.5 mg, 290 U, see above), NAD (31 mg, 0.04 mmol), buffer (12.8 mL of 0.1 M sodium phosphate pH 8.0), and DMF (1.6 mL). After work up, the residue was analysed by TLC and separated by HPLC. Preparative HPLC was achieved using a μBondapak radial compression reverse phase C_8, 25 × 100 mm, 300 Å, 15-20 μm column (Millipore Waters, Mississauga, ON) ran at 10 mL/min. The gradient started at 5% acetonitrile in water for 5 min, then went from 5% to 60% over 20 min, from 60% to 95 % over 5 min, and stayed at 95% for 10 min. The UV detector was set at 220 nm. Of the ten peaks collected, none displayed NMR data consistent with the expected Diels-Alder adduct structure.

Tyrosinase reaction with 2-(4-penten-1-yl)phenol (82). Mushroom tyrosinase (monophenol monooxygenase; polyphenol oxidase; catechol oxidase, monophenol, dihydroxyphenylalanine:oxygen oxidoreductase; EC 1.14.18.1) had an activity of 3400 units/mg solid. Unit definition: One unit will cause an increase in absorbance at 280 nm of 0.001 per minute at pH 6.5 and 25 °C in a 3 mL reaction mixture containing L-

tyrosine. The enzymatic reaction was based on the methods described by Müller and Waldmann,[92] and by Burton and coworkers.[140] The enzyme was immobilized on glass beads as follows. The beads (1.2 g, 106 microns and finer, Sigma) were washed with buffer (0.05 M phosphate buffer, pH 7.0) and dried under high vacuum. A solution of enzyme (8 mg, 27 kU) in buffer (0.44 mL) was poured over the dry glass beads. The mixture was dried under a gentle air stream with occasional mixing.). An enzyme assay was prepared by mixing phenol (50 mg, 0.53) or catechol (50 mg, 0.45 mmol), with immobilized tyrosinase (0.58 g, 13 kU), dry chloroform (30 mL) and buffer (0.1 mL). The enzyme was found to be active under these conditions. A blank reaction mixture contained compound **82** (30 mg, 0.2 mmol), dry chloroform (30 mL), and buffer (0.1 mL). The enzymatic reaction was started by the addition of immobilized tyrosinase (0.58 g, 13 kU) to a solution of substrate **82** (30 mg, 0.2 mmol) in dry chloroform (30 mL) and buffer (0.1 mL). The reaction mixtures were left on an orbital shaker under normal atmosphere at r.t. and 100 rpm, and followed by TLC for 16 days. The enzyme was filtered and washed with chloroform. The filtrate was dried ($MgSO_4$). Evaporation of the solvent *in vacuo* afforded pure recovered starting material **82** for both the blank (21 mg, 75%) and the enzymatic reaction (20 mg, 70%).

Tyrosinase reaction with 4-(4-penten-1-yl)phenol (92). Compound **92** was tested with tyrosinase as described above for compound **82**. Therefore, the enzymatic reaction was started by the addition of immobilized tyrosinase (4.5 g, 13 kU) to a solution of substrate **92** (19 mg, 0.12 mmol) in dry chloroform (12 mL) and buffer (0.02 mL). Work up afforded pure recovered material **92** for the blank (5 mg, quant.) and uncyclized *o*-quinone product partially polymerized (19 mg, quant.).

Tyrosinase reaction with 4-(4-penten-1-yl)phenol (92) in presence of ethyl vinyl ether. The immobilized mushroom tyrosinase was prepared and assayed as for

the testing of compound **82**. The blank reaction was a mixture of compound **92** (6 mg, 0.037 mmol), chloroform (6 mL), buffer (0.04 mL), and ethyl vinyl ether (6 mL, 62.8 mmol). The enzymatic reaction contained the substrate **92** (25 mg, 0.154 mmol), chloroform (6 mL), buffer (0.04 mL), ethyl vinyl ether (6 mL, 62.8 mmol), and immobilized enzyme (10 g, 255 kU). Both mixtures were left on an orbital shaker at r.t. and 150 rpm for one day, then at 175 rpm for 15 days. At that point, the enzymatic reaction contained mostly unchanged starting material **92** (from TLC). More immobilized enzyme was added (1.25 g, 29.3 kU), as well as more buffer (0.02 mL), and more ethyl vinyl ether (1.5 mL, 15.7 mmol). The reaction was shaken at 175 rpm for one week, at which time the control had started to show some decomposition. The blank reaction was evaporated *in vacuo* and found to contain mostly starting material **92**. The enzymatic reaction was filtered and the enzyme was washed with chloroform. The filtrate was evaporated *in vacuo* and the residue purified by silica gel flash column chromatography (0 to 20% diethyl ether in petroleum ether). The three TLC spots were two different polymers (R_f 0.54 and 0.92 with 50% diethyl ether in petroleum ether) and starting material **92** (12 mg, 50%, R_f 0.67 with 50%).

Tyrosinase reaction with 4-(5-hexen-1-yl)phenol (93). Compound **93** was tested with tyrosinase as described above for compound **92**. Thus, the enzymatic reaction was started by the addition of immobilized tyrosinase (5 g, 59 kU, see above) to a solution of substrate **93** (20 mg, 0.11 mmol) in dry chloroform (20 mL) and buffer (0.03 mL). Work up afforded pure recovered **93** for the blank (5.5 mg, 90%), and a mixture of the uncyclized *o*-quinone product partially polymerized (18 mg, 90%) for the enzymatic reaction. The latter underwent further polymerization when purification was attempted. As shown in the compounds section for **120**, the *o*-quinone could be trapped by its Diels-Alder reaction with ethyl vinyl ether to give compound **120**.

Tyrosinase reaction with 4-(6-hepten-1-yl)phenol (94). Compound **94** was tested with tyrosinase as described above for compound **92**. Therefore, the enzymatic reaction was started by adding immobilized tyrosinase (5 g, 59 kU) to a solution of substrate **94** (20 mg, 0.11 mmol) in dry chloroform (20 mL) and buffer (0.03 mL). Work up afforded pure recovered starting material **94** for the blank (5.5 mg, 90%), and a mixture of the uncyclized *o*-quinone product partially polymerized (20 mg, quant.) for the enzymatic reaction. The latter underwent further polymerization when purification was attempted.

Tyrosinase reaction with 4-(6-hepten-1-yl)phenol (94) in presence of ethyl vinyl ether. Mushroom tyrosinase (EC 1.14.18.1, activity of 3400 units/mg solid) was immobilized on glass beads as described for 2-(4-penten-1-yl)phenol **92**. The 106 microns and finer glass beads (10.9 g) were washed with buffer (0.05 M phosphate buffer, pH 7.0) and dried under high vacuum. A solution of enzyme (82 mg, 129 kU) in buffer (4 mL) was poured over the dry glass beads and dried. A blank reaction mixture was prepared by mixing 4-(6-hepten-1-yl)phenol (**94**) (6 mg, 0.032 mmol) with dry chloroform (3 mL) and buffer (0.005 mL). The enzymatic reaction was started by adding immobilized tyrosinase (5 g, 59 kU) to a solution of substrate **94** (20 mg, 0.10 mmol) in dry chloroform (20 mL) and buffer (0.03 mL). The reaction mixtures were left on an orbital shaker at r.t. and 150 rpm for two days. Immobilized enzyme (9.5 g, 245 kU) and buffer (0.01 mL) were added and the mixture was left on the shaker at r.t. and 100 rpm for 3 more days (time required to react all compound **94**). Ethyl vinyl ether was then added to both the blank (5 mL) and the enzymatic reaction (10 mL). After one day at 100 rpm and r.t., the reactions were worked up. Evaporation of the solvent afforded pure recovered material **94** for the blank (5.0 mg, 85%). For the enzymatic reaction, the enzyme was filtered off and washed with chloroform, affording polymerized product.

REFERENCES

(1) Reviews: (a) Stauton, J.; Wilkinson, B. In *Biosynthesis Polyketides and Vitamins; The Biosynthesis of Aliphatic Polyketides; Topics in Current Chemistry*, Vol. 195; Leeper, I. J.; Vederas, J. C. Eds.; Springer Verlag Berlin Heidelberg: Cambridge, 1998; pp 49-92. (b) Cane, D. E.; Walsh, C. T.; Khosla, C. *Science* **1998**, *282*, 63-68. (c) Hopwood, D. A. *Chem. Rev.* **1997**, *97*, 2465-2497. (d) Hutchinson, C. R.; Fujii, I. *Annu. Rev. Microbiol.* **1995**, *49*, 201-238. (e) Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 881-885. (f) Katz, L.; Donadio, S. *Annu. Rev. Microbiol.* **1993**, *47*, 875-912. (g) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37-66. (h) Eckermann, S.; Schröder, G.; Schmidt, J.; Strack, D.; Edrada, R. A.; Helariutta, Y.; Elomaa, P.; Kotilainen, M.; Kilpeläinen, I.; Proksch, P.; Teeri, T. H.; Schröder, J. *Nature* **1998**, *396*, 387-390. (i) Schröder, J. *Nature Struct. Biol.* **1999**, *6*, 714-716. (j) Ferrer, J. L.; Jez, J. M.; Bowman, M. E., Dixon, R. A., Noel, J. P. *Nature Struct. Biol.* **1999**, *6*, 775-784.

(2) (a) Mann, J. In *Secondary Metabolism*, 2nd Ed.; Oxford Science Publications; Clarendon Press: Oxford, 1997; pp 1-94. (b) Herbert, C. R. In *The Biosynthesis of Secondary Metabolites*, 2nd Ed.; Chapman and Hall Ed.: New York, 1989; pp 16-62

(3) Li, Z.; Martin, F. M.; Reese, P. B.; Yoshizawa, Y.; Vederas, J. C. In *Biosynthesis and Metabolism of Secondary Metabolites Natural Products*, Petroski, R. J. and McCormick, S. P. Eds.; Plenum: New York; 1992; pp 27-39.

(4) (a) Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212-3213. (b) Li, Z.; Martin, F. M.; Vederas, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1531-1533.

(5) Liu, Y.; Li, Z.; Vederas, J. C. *Tetrahedron* **1998**, *54*, 15937-15958.

(6) Tsantrizos, Y. S.; Zhou, F.; Famili, P.; Yang, X. *J. Org. Chem.* **1995**, *60*, 6922-6929.

(7) Jacobs, A.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1995**, 863-864.

(8) Gokhale, R. S.; Lau, J.; Cane, D. E.; Khosla, C. *Biochemistry*, **1998**, *37*, 2524-2528.

(9) (a) Shen, B.; Hutchinson, C. R. *Science* **1993**, *262*, 1535-1540. (b) Shen, B.; Summers, R. G.; Gramajo, H.; Bibb, M. J.; Hutchison, C. R. *J. Bacteriol.* **1992**, *174*, 3818-3821.

(10) (a) Bao, W.; Sheldon, P. J.; Wendt-Pienkowski, E.; Hutchinson, C. R. *J. Bacteriol.* **1999**, *181*, 4690-4695. (b) Bao, W.; Sheldon, P. J.; Hutchinson, C. R. *Biochemistry* **1999**, *38*, 9752-9757.

(11) Beck, J.; Ripka, S.; Siegner, A.; Schiltz, E.; Schweizer, E. *Eur. J. Biochem.* **1990**, *192*, 487-498.

(12) Offenzeller, M.; Su, Z.; Santer, G.; Moser, H.; Traber, R.; Memmer, K.; Schneider-Scherzer, E. *J. Biol. Chem.* **1993**, *268*, 26127-26134.

(13) (a) Kao, M.; Luo, G.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1994**, *116*, 11612-11613. (b) Cortes, J.; Wiesmann, K. E. H.; Roberts, G. A.; Brown, M. J. B.; Staunton, J.; Leadlay, P. F. *Science* **1995**, *278*, 1487-1489. (c) Kao, M.; Luo, G.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1995**, *117*, 9105-9106. (d) Kao, M.; Luo, G.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1996**, *118*, 9184-9185.

(14) (a) Kuhstoss, S.; Huber, M.; Turner, J. R.; Paschal, J. W.; Rao, R. N. *Gene* **1996**, *183*, 231-236. (b) Oliynyk, M.; Brown, M. J. B.; Cortes, J.; Staunton, J.; Leadlay, P. F. *Chem. Biol.* **1996**, *3*, 833-839.

(15) McDaniel, R.; Kao, C. M.; Fu, H.; Hevezi, P.; Guståfsson, C.; Betlach, M.; Ashley, G.; Cane, D. E.; Khosla, C. L. *J. Am. Chem. Soc.* **1997**, *119*, 4309-4310.

(16) (a) Lowden, P. A. S.; Böhm, G. A.; Staunton, J.; Leadlay, P. L. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2249-2251. (b) Otsuka, M.; Eguchi, T.; Shindo, K.; Kakinuma, K. *Tetrahedron Lett.* **1998**, *39*, 3185-3188.

(17) Jacobsen, J. R.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1998**, *120*, 9096-9097.

(18) Fernandez-Moreno, M. A.; Martinez, E.; Boto, L.; Hopwood, D. A.; Malpartida, F. *J. Biol. Chem.* **1992**, *267*, 19278-19290.

(19) Lomovskaya, N.; Doi-Katayama, Y.; Filippini, S.; Nastro, C.; Fonstein, L.; Gallo, M.; Colombo, A. L.; Hutchinson, C. R. *J. Bacteriol.* **1998**, *180*, 2379-2386.

(20) Shen, B.; Hutchinson, C. R. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6600-6604.

(21) Shen, B.; Hutchinson, C. R. *Biochemistry* **1993**, *32*, 11149-11154.

(22) Kendrew, S. G.; Katayama, K.; Deutsch, E.; Madduri, K.; Hutchinson, C. R. *Biochemistry* **1999**, *38*, 4794-4799.

(23) Gokhale, R. S.; Tsuji, S. Y.; Cane, D. E.; Khosla, C. *Science* **1999**, *284*, 482-485.

(24) (a) Hutchinson, C. R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3336-3338. (b) McDaniel, R. M.; Thamchaipenet, A.; Gustafsson, C.; Fu, H.; Betlach, M.; Betlach, M.; Ashley, G. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1846-1851. (c) Shen, Y.; Yoon, P.; Yu, T.-W.; Floss, H. G.; Hopwood, D.; Moore, B. S. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3622-3627.

(25) (a) Grundy, S. M.; Vega, G. L. *J. Lipids Res.* **1985**, *26*, 1464-1475. (b) Brown, M. S.; Faust, J. R.; Goldstein, J. L.; Kaneko, I.; Endo, A. *J. Biol. Chem.* **1978**, *253*, 1121-1128. (c) Endo, A.; Hasumi, K. *Nat. Prod. Rep.* **1993**, *10*, 541-550.

(26)Rogers, C. H.; Panini, S. R.; Rudney, H. In *3-Hydroxymethylglutaryl Coenzyme A Reductase*; Sabine, J. R. Ed.; CRC Press Inc.: Florida, 1983; pp 58-75.

(27) (a) Endo, A.; Kuroda, M.; Tanzawa, K. *FEBS Lett.* **1976**, *72*, 323-326. (b) Alberts, A. W. *Am. J. Cardiol.* **1988**, *62*, 10J-15J.

(28) Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiotics* **1976**, *29*, 1346-1348.

(29) Brown, A. G.; Smale, T. C.; King, Y. J.; Hasenkamp, R.; Thomson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165-1170.

(30)Endo, A.; Hasumi, K. *J. Antibiot.* **1979**, *32*, 852-854.

(31) (a) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 3957-3961. (b) Brown, A. G.; Smale, T. C. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165-1170.

(32) Reviews: (a) Chapleur, Y. *Recent Prog. Chem. Synth. Antibiot. Relat. Microb. Prod.* **1993**, 829-937. (b) Rosen, T. *Stud. Nat. Prod.* **1993**, *13*, 553-627.

(33) Review: Clive, D. L. J.; Murthy, K. S. K. Wee, A. G. H.; Prasad, J. S.; Da Silva, G. V. J.; Majewski, M.; Anderson, P. C. *Stud. Nat. Prod. Chem.* **1992**, *11*, 335-377.

(34) (a) Review: Rosen, T.; Heathcock, C. H. *Tetrahedron* **1986**, *42*, 4909-4954. (b) Yang, Y.-L.; Manna, S.; Falck, J. *J. Am. Chem. Soc.* **1984**, *106*, 3811-3814. (c) Davidson, A. H.; Jones, A. J.; Floyd, C. D.; Lewis, C.; Myers, P. L. *J. Chem. Soc., Chem Commun.* **1987**, 1786-1787. (d) Hecker, S. J.; Heathcock, C. H. *J. Am. Chem. Soc.* **1986**, *108*, 4586-4594. (e) Falck, J. R.; Yang, Y.-L. *Tetrahedron Lett.* **1984**, *25*, 3563-3566. (f) Clive, D. L. J.; Murthy, K. S. K.; George, R.; Poznansky, M. J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2099-2108. (g) Hagiwara, H.; Kon-no, M.; Nakano, T.; Uda, H. *J. Chem. Soc., Perkin Trans. 1* **1994**, 417-2430. (h) Clive, D. L. J.; Wickens, P. L.; da Silva, G. V. J. *J. Org. Chem.* **1995**, *60*, 5532-5536. (i) Araki, Y.; Konoike, T. *J. Org. Chem.* **1997**, *62*, 5299-5309.

(35) (a) Jendralla, H.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Kerekjarto, B.; Kesseler, K.; Krause, R.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 61-70. (b) Vrabe, H.; Matsuka, T.; Sato, F. *Tetrahedron Lett.* **1992**, *33*, 4183-4186. (c) Miyachi, N.; Yanagawa, Y.; Iwasaki, H.; Ohara, Y.; Hiyama, T. *Tetrahedron Lett.* **1993**, *34*, 8267-8270. (d) Matsumoto, M.; Yamaura, T. *Heterocycles* **1994**, *38*, 2589-2592. (e) Novák, L.; Hornyánsky, G.; Rohály, J.; Kolonits, P.; Szantay, C. *Liebigs Ann.* **1995**, 1877-1883.

(36) (a) Berk, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. V.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 52-60. (b) Sliskovic, D. R.; Picard, J. A.; Roark, W. H.; Roth, B. D.; Ferguson, E.; Krause, B. R.; Newton, R. S.; Sekerle, C.; Shaw, M. K. *J. Med. Chem.* **1991**, *34*, 367-373. (c) Roth, B. D.; Boca, T. M. A.; Blankley, C. J.; Chucholowski, A. W.; Creger, P. L.; Creswell, M. W.; Ferguson, E.; Newton, R. S.; Brien, P. O.; Picard, J. A.; Roark, W. H.; Sekerle, C. S.; Sliskovic, D. R.; Wilson, M. W. *J. Med. Chem.* **1991**, *34*, 463-466.

(37) Review: Christians, U.; Jacobsen, W.; Floren, L. C. *Pharmacol. Ther.* **1998**, *80*, 1-34.

(38) Endo, A. *J. Lipid Res.* **1992**, *33*, 1569-1582.

(39) Bilheimer, D. W.; Grundy, S. M.; Brown, M. S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 4124-4128.

(40) (a) Stein, E. A. *Atherosclerosis*, **1994**, *108*, S105-S116. (b) Willis, R. A.; Folkers, K.; Tucker, J. L.; Ye, C.-Q.; Xia, L.-J.; Tamagawa, H. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 8928-8930. (c) Folkers, K.; Langjoen, P.; Willis, R.; Richardson, P.; Xia, L.-J.; Ye, C.-Q.; Tamagawa, H. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 8931-8934. (d) Amin, D.; Rutledge, R. Z.; Needle, S. J.; Hele, D. J.; Neuenswander, K.; Bush, R. C.; Bilder, G. E.; Perrone, M. H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1996**, *353*, 233-240.

(41) Review: (a) Abe, I.; Tomesh, J. C.; Wattanasin, S.; Prestwich, G. D. *Nat. Prod. Rep.* **1994**, *11*, 293-302. (b) Gotteland, J.-P.; Brunelle, I.; Gendre, F.; Désiré, J.; Delhon, A.; Junquéro, D.; Oms, P.; Halazy, S. J. *J. Med. Chem.* **1995**, *38*, 3207-3216.

(42)(a) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694-3701. (b) Greenspan, M. D.; Yudkovitz, J. B. *J. Bacteriol.* **1985**, *162*, 704-707. (c) Endo, A.; Negishi, Y.; Iwashita, T.;

Mizukawa, K.; Hirama, M. *J. Antibiot.* **1985**, *38*, 444-448. (d) Shiao, M.-S.; Don, H.-S. *Proc. Natl. Sci. Counc. Repub. China [B]* **1987**, *11*, 223-231. (e) Yoshizawa, Y.; Witter, D. J.; Liu, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 2693-2694. (f) Wagschal, K.; Yoshizawa, Y.; Witter, D. J.; Liu, Y.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2357-2363.

(43) Snyder, W. C.; Reinhart Jr., K. L. *J. Am. Chem. Soc.* **1984**, *106*, 787-789.

(44)(a) Kimura, K.; Komagata, D.; Murakawa, S.; Endo, A. *J. Antibiotics* **1990**, *43*, 1597-1600. (b) Nakamura, T.; Komagata, S.; Sakai, K.; Endo, A. *J. Antibiotics* **1990**, *43*, 1621-1622. (c) Komagata, D.; Shimada, H.; Murakawa, S.; Endo, A. *J. Antibiotics* **1989**, *42*, 407-412. (d)Endo, A.; Hasumi, K. *J. Antibiotics* **1985**, *38*, 321-327.

(45) Treiber, L. R.; Reamer, R. A.; Rooney, C. S.; Ramjit, H. G. *J. Antibiotics* **1989**, *42*, 30-36.

(46)Witter, D. J.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613-2623.

(47) Reviews: (a) Craig, D. *Chem. Soc. Rev.* **1987**, *16*, 187-238. (b) Fallis, A. G. *Can. J. Chem.* **1984**, *62*, 3182-3234. (c) Roush, W. R. In *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, F.; Paquette, L. A., Eds.; Pergamon: Oxford, 1991; Vol 5, pp 513-550. (d) Deslongchamps, P. *Pure & Appl. Chem.* **1992**, *64*, 1831-1847.

(48) Morrison, R. T.; Boyd, R. N. In *Organic Chemistry*, 5th Ed.; Allyn and Bacon, Inc.: Boston; 1987; pp 1149-1166.

(49) (a) Ichihara, A. *Synthesis* **1987**, 207-222. (b) Kiselev, V. D.; Konovalov, A. I. *Russ. Chem. Rev.* **1989**, *58*, 230-249. (c) Shibasaki, M.; Sasai, H.; Arai, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1237-1256. (d) Dell, C. P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3873-3905.

(50) Review: Oppolzer, W. In *Comprehensive Organic Synthesis*; ; Trost, B. M.; Fleming, F.; Paquette, L. A., Eds.; Pergamon: Oxford, 1991; Vol 5, pp 315-399.

(51) Reviews: (a) Bols, M.; Skrydstrup, T. *Chem. Rev.* **1995**, *95*, 1253-1277. (b) Fensterbank, L.; Malacia, M.; Sieburth, S. M. *Synthesis* **1997**, 813-854. (c) Gauthier Jr., D. R.; Zandi, K. S.; Shea, K. J. *Tetrahedron* **1998**, *54*, 2289-2338.

(52) (a) Ainsworth, P. J.; Craig, D.; Reader, J. C.; Slawin, A. M. Z.; White, A. J. P.; Williams, D. J. *Tetrahedron* **1995**, *51*, 11601-11621. (b) Ainsworth, P. J.; Craig, D.; White, A. J. P.; Williams, D. J. *Tetrahedron*, **1996**, *52*, 8937-8946. (c) Ainsworth, P. J.; Craig, D.; Reader, J. C.; Slawin, A. M. Z.; White, A. J. P. *Tetrahedron*, **1996**, *52*, 695-724. (d) Craig, D.; Ford, M. J.; Stones, J. A. *Tetrahedron Lett.* **1996**, *37*, 535-538.

(53) Batey, R. A.; Thadani, A. N.; Lough, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 450-451.

(54) Review: Pinder, U.; Lutz, G.; Otto, C. *Chem. Rev.* **1993**, *93*, 741-761.

(55) Yates, P.; Eaton, P. *J. Am. Chem. Soc.* **1960**, *82*, 4436-4437.

(56) Breslow, R.; Rideout, D. C. *J. Am. Chem. Soc.* **1980**, *102*, 7816-7817.

(57) (a) Otto, S.; Blokzijl, W.; Engberts, J. B. F. N. *J. Org. Chem.* **1994**, *59*, 5372-5376. (b) Engberts, J. B. F. N. *Pure Appl. Chem.* **1995**, *67*, 823-828. (c) Grieco, P. A. In *Organic Synthesis in Water*; Blacky Academic and Professional: London; 1998.

(58) (a) Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 7430-7432. (b) Blake, J. F.; Lim, D.; Jorgensen, W. L. *J. Org. Chem.* **1994**, *59*, 803-805. (c) Furlani, T. R.; Gao, J. *J. Org. Chem.* **1996**, *61*, 5492-5497.

(59) (a) Breslow, R.; Zhu, Z. *J. Am. Chem. Soc.* **1995**, *117*, 9923-9924. (b) Sangwan, N. K.; Schneider, H.-J. *J. Am. Chem. Soc., Perkin Trans. 1* **1989**, 1223-1227.

(60) van der Wel, G. K.; Wijnen, J. W.; Engberts, J. B. F. N. *J. Org. Chem.* **1996**, *61*, 9001-9005.

(61) (a) Wijnen, J. W.; Engberts, J. B. F. N. *J. Org. Chem.* **1997**, *62*, 2039-2044. (b) Wijnen, J. W.; Engberts, J. B. F. N. *Liebigs Ann.* **1997**, 1085-1088.

(62) (a) Brandes, E. B.; Grieco, P. A.; Gajewski, J. J. *J. Org. Chem.* **1989**, *54*, 515-516. (b) Grieco, P. A.; Brandes, E. B.; McCann, S.; Clark, J. D. *J. Org. Chem.* **1989**, *54*, 5849-5851. (c) Severance, D. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1992**, *114*, 10966-10968. (d) Gao, J. *J. Am. Chem. Soc.* **1994**, *116*, 1563-1564. (e) Gajewski, J. J.; Brichford, N. L. *J. Am. Chem. Soc.* **1994**, *116*, 3165-3166. (f) Gajewski, J. J. *Acc. Chem. Res.* **1997**, *30*, 219-225.

(63) Lubineau, A. *J. Org. Chem.* **1986**, *51*, 2142-2144.

(64) (a) Kool, E. T.; Breslow, R. *J. Am. Chem. Soc.* **1988**, *110*, 1596-1597. (b) Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 159-164.

(65) Johannsen, M.; Jorgensen, K. A. *Tetrahedron* **1996**, *52*, 7321-7328.

(66) (a) Dignam, K. J.; Hegarty, A. F.; Quain, P. L. *J. Org. Chem.* **1978**, *43*, 388-393. (b) Inoue, I.; Araki, K.; Shiraishi, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3079-3083. (c) Rohloff, J. C.; Robinson, J.; Gardner, J. O. *Tetrahedron Lett.* **1992**, *33*, 3113-3116. (d) Wijen, J. W.; Steiner, R. A.; Enberts, J. B. N. F. *Tetrahedron Lett.* **1995**, *35*, 5389-5392.

(67) (a) Otto, S.; Bertoncin, F.; Engberts, J. B. N. F. *J. Am. Chem. Soc.* **1996**, *118*, 7702-7707. (b) Otto, S.; Engberts, J. B. N. F.; Kwak, J. C. T. *J. Am. Chem. Soc.* **1998**, *120*, 9517-9525.

(68) Zhu, Z.; Espenson, J. H. *J. Am. Chem. Soc.* **1997**, *119*, 3507-3512.

(69) Review: Corey, E. J.; Guzman-Perez, A. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 388-401.

(70) (a) Ishitani, H.; Kobayashi, S. *Tetrahedron Lett.* **1996**, *37*, 7357-7360. (b) Saito, T.; Kawamura, M.; Nishimuraa, J.-I. *Tetrahedron Lett.* **1997**, *38*, 3231-3234.

(71) (a) Kanemasa, S.; Oderaotoshi, Y.; Yamamoto, H.; Tanaka, J.; Wada, E. *J. Org. Chem.* **1997**, *62*, 6454-6455. (b) Otto, S.; Bocaletti, G.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1998**, *120*, 4238-4239.

(72) (a) Giguère, R. J.; Bray, T. L.; Duncan, S. M. *Tetrahedron Lett.* **1986**, *27*, 4945-4948. (b) Garrigues, B.; Laporte, C.; Laurent, R.; Laporterie, A.; Dubac, J. *Liebigs Ann.* **1996**, 739-741.

(73) Bouaziz, Z.; Nebois, P.; Fillion, H.; Luche, J. L.; Jenner, G. *Tetrahedron* **1995**, *51*, 4057-4064.

(74) (a) Katritzky, A. R.; Allin, S. M. *Acc. Chem. Res.* **1996**, *29*, 399-406. (b) Korzenski, M. B.; Kollis, J. W. *Tetrahedron Lett.* **1997**, *38*, 5611-5614.

(75) Fischer, T.; Sethi, A.; Welton, T.; Woolf, J. *Tetrahedron Lett.* **1999**, *40*, 793-796.

(76) Endo, K.; Koike, T.; Sawaki, T.; Hayashida, O.; Masuda, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 4117-4122.

(77) (a) Grieco, P. A.; Handy, S. T.; Beck, J. P. *Tetrahedron Lett.* **1994**, *35*, 2663-2666. (b) Grieco, P. A.; Beck, J. P.; Handy, S. T.; Saito, N.; Daeuble, J. F. *Tetrahedron Lett.* **1994**, *35*, 6783-6786. (c) Grieco, P. A.; Kaufman, M. D.; Daeuble, J. F.; Saitto, N. *J. Am. Chem. Soc.* **1996**, *118*, 2095-2096.

(78) Diego-Castro, M. J.; Hailes, H. C. *Tetrahedron Lett.* **1998**, *39*, 2211-2214.

(79) (a) Kang, J.; Rebek Jr., J. *Nature* **1997**, *385*, 50-52. (b) Kang, J.; Hilmersson, G.; Santamaría, J.; Rebek Jr., J. *J. Am. Chem. Soc.* **1998**, *120*, 3650-3656. (c) Kang, J.; Santamaría, J.; Himersson, G.; Rebek Jr., J. *J. Am. Chem. Soc.* **1998**, *120*, 7389-7390.

(80) Colonna, S.; Manfredi, A.; Annunziata, R. *Tetrahedron Lett.* **1988**, *29*, 3347-3350.

(81) Review: Blackburn, G. M.; Datta, A.; Denham, H.; Wentworth Jr., P. *Adv. Phy. Org. Chem.* **1998**, *31*, 249-392.

(82) (a) Seelig, B.; Jäschke, A. *Tetrahedron Lett.* **1997**, *38*, 7729-7732. (b) Tarasow, T. M.; Tasarow, S. L.; Eaton, B. E.; *Nature* **1997**, *389*, 54-57. (c) Frauendorf, C.; Jäschke, A. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1378-1381. (d) Tarasow, T. M.; Eaton, B. E. *Biopolymers (Nucleic Acid Sciences)* **1998**, *48*, 29-37. (e)

Tarasow, T. M.; Tarasow, S. L.; Tu, C.; Kellogg, E.; Eaton, B. E. *J. Am. Chem. Soc.* **1999**, *121*, 3614-3617.

(83) Reviews: (a) Laschat, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 289-291. (b) Ichihara, A.; Oikawa, H. In *Biological Diels-Alder Reaction in Biosynthesis of Phytotoxins*; Ogura, K.; Sankawa, U. Eds.; Kodansha, Harwood Academic Publishers: Tokyo, 1997; pp 119-144. (c) Ichihara, A.; Oikawa, H. *Curr. Org. Chem.* **1998**, *2*, 365-394.

(84) Review: (a) Ichihara, A.; Oikawa, H. *Biosci. Biotech. Biochem.* **1997**, *61*, 12-18. (b) Oikawa, H.; Yokota, T.; Sakano, C.; Suzuki, Y.; Naya, A.; Ichihara, A. *Biosci. Biotech. Biochem.* **1998**, *62*, 20166-2022. (c) Katayama, K.; Kobayashi, T.; Oikawa, H.; Honma, M.; Ichihara, A. *Biochim. Biophys. Acta* **1998**, *1384*, 387-395. (d) Oikawa, H.; Suzuki, Y.; Katayama, K.; Naya, A.; Sakano, C.; Ichihara, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1225-1232. (e) Ichihara, A.; Oikawa, H. In *Comprehensive Natural Products Chemistry*, Vol 1 *Polyketides and Other Secondary Metabolites*; Sankawa U. Ed.: New York, 1999 pp 367-408.

(85) Ichihara, A.; Tazaki, H.; Sakamura, S. *Tetrahedron Lett.* **1983**, *24*, 5373-5376.

(86) (a) Simpson, T. J. *Annu. Rep. Chem. Soc.* **1986**, 353-357. (b) Oikawa, H.; Kobayashi, T.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748-8756.

(87) Alam, S. S.; Bilton, J. M.; Slawin, Z. M.; Williams, D. J.; Sheppard, R. N.; Strange, R. M. *Phytochemistry*, **1989**, *28*, 2627-2628.

(88) (a) Oikawa, H.; Yokota, T.; Abe, T.; Ichihara, A.; Sakamura, S.; Yoshizawa, Y.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1282-1284. (b) Oikawa, H.; Yokota, T.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1989**, 1284-1285.

(89) Oikawa, H.; Suzuki, Y.; Naya, A.; Katayama, K.; Ichihara, A. *J. Am. Chem. Soc.* **1994**, *116*, 3605-3606.

(90) (a) Oikawa, H.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Chem. Soc., Chem. Commun.* **1995**, 1321-1322. (b) Oikawa, H.; Kobayashi, T.; Katayama, K. Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748-8756.

(91) Reviews: (a) Sánchez-Ferrer, A.; Rodríguez-López, J. N.; García-Cánovas, F.; García-Carmona, F. *Biochim. Biophys. Acta* **1995**, *1247*, 1-11.

(92) Müller, G. H.; Waldmann, H. *Tetrahedron Lett.* **1996**, *37*, 3833-3836.

(93) Hendrickson, L.; Davis, C. R.; Roach, C.; Nguyen, D. K.; Aldrich, T.; McAda, P. C.; Reeves, C. D. *Chem. Biol.* **1999**, *6*, 429-439.

(94) Kennedy, J.; Auclair, K.; Kendrew, S. G.; Park, C.; Vederas, J. C.; Hutchinson, C. R. *Science* **1999**, *284*, 1368-1372.

(95) Reviews: (a) Schultz, H. *Biochim. Biophys. Acta* **1991**, *1081*, 109-120. (b) Bremer, J.; Osmundsen, H. In *Fatty Acids Metabolism and Its Regulation*; Numa, S. Ed.; Elsevier: New York, 1984; pp 113-154.

(96) (a) Cerdan, S.; Künnecke, B.; Dölle, A.; Seelig, J. *J. Biol. Chem.* **1988**, *263*, 11664-11674. (b) Suzuki, H.; Yamada, J.; Watanabe, T.; Suga, T. *Biochim. Biophys. Acta* **1989**, *990*, 25-30. (c) Bergseth, S.; Poisson, J.-P.; Bremer, J. *Biochim. Biophys. Acta* **1990**, *1042*, 182-187.

(97) Liu, Y. *Biosynthetic Studies on Dehydrocurvularin, and Modification of the Swern Oxidation*, Ph. D. Thesis, University of Alberta, 1996.

(98) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522-524.

(99) Lee, M. S.; Qin, G.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1989**, *111*, 6234-6241.

(100) Yamada, S.; Yokoyama, Y.; Shioiri, T. *J. Org. Chem.* **1974**, *39*, 3302-3303.

(101) Kunau, W.-H.; Bühne, S.; De La Garza, M.; Kionka, C.; Mateblowski, M.; Schultz-Borchard, U.; Thieringer, R. *Biochem. Soc. Trans.* **1988**, *16*, 418-420.

(102) (a) Villanueva, J. R.; Acha, I. G. *Methods Microbiol.* **1971**, *4*, 665-671. (b) Ventura, L.; Ramòn, D. *FEMS Microbiol. Lett.* **1991**, *82*, 189-194. (c) Solis, S.; Flores, M. E.; Huitron, C. *Lett. Appl. Microbiol.* **1996**, *23*, 36-42.

(103) Meiners, S.; Gharyal, P. K.; Schlinder, M. *Planta* **1991**, *184*, 443-447.

(104) Patzelt, H.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1993**, 1258-1260.

(105) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253-1255.

(106) Reviews: (a) Akhtar, M.; Wright, J. N. *Nat. Prod. Rep.* **1991**, *8*, 527-551. (b) Ortiz de Montellano, P. R. In *Cytochrome P450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano Ed.; Plenum: New York, 1986.

(107) (a) Correia, M. A.; Ortiz de Montellano, P. R. In *Medical Implications in Cytochrome P450 Catalyzed Transformations*; Ruckpaul, K.; Rein, H. Eds.; Frontiers in Biotransformation, 1993, Vol 8, Chapter 3. (b) Coulson, C. J.; King, D. J.; Wiseman, A. *Trends Biochem. Sci.* **1984**, *10*, 446-449.

(108) (a) Oikawa, H.; Ichihara, A.; Sakamura, S. J. *J. Chem. Soc., Chem. Commun.* **1988**, 600-602. (b) Oikawa, H.; Ichihara, A.; Sakamura, S. J. *J. Chem. Soc., Chem. Commun.* **1990**, 908-909.

(109) (a) Oikawa, H.; Mukarami, Y.; Ichihara, A. *Tetrahedron Lett.* **1991**, *32*, 4533-4536. (b) Oikawa, H.; Mukarami, Y.; Ichihara, A. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2949-2953.

(110) See Experimental section for crystal data. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center (CCDC).

(111) Personal communication and Watanabe, C. M. H.; Townsend, C. A. *J. Am. Chem. Soc.* **1998**, *120*, 6231-6239.

(112) Cooper, T. G. In *The Tools of Biochemistry*; John Wiley and Sons, Inc: New York, 1977.

(113) Reviews: (a) Wong, C.-H. *Science* **1989**, *244*, 1145-1151. (b) Turner, N. J. *Nat. Prod. Rep.* **1994**, *11*, 1-15. (c) Mori, K. *Synlett* **1995**, 1097-1109. (d) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769-3826. (e) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 157-169.

(114) Dickens, M. L.; Ye, J.; Strohl, W. R. *J. Bacteriol.* **1995**, *177*, 536-543.

(115) Jacobsen, J. R.; Keatinge-Clay, A. T.; Cane, D. E.; Khosla, C. *Bioorg. Med. Chem.* **1998**, *6*, 1171-1177.

(116) Fu, H.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *J. Am. Chem. Soc.* **1994**, *116*, 6443-6444.

(117) Meurer, G.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1995**, *117*, 5899-5900.

(118) Hunziker, D.; Yu, T.-W.; Hutchinson, C. R.; Floss, H. G.; Khosla, C. *J. Am. Chem. Soc.* **1998**, *120*, 1092-1093.

(119) Wong, C.-H. *Science* **1989**, *244*, 1145-1151.

(120) International Union of Biochemistry, *Enzyme Nomenclature*; Academic Press: Orlando, 1984.

(121) Fershy, A. In *Enzyme Structure and Mechanism*, 2nd Ed.; Freeman Publications: San Francisco, 1985.

(122) Reviews: (a) Brink, L. E. S.; Tramper, J.; Luyben, K. C. A. M.; Van't Riet, K. *Enzyme Microb. Technol.* **1988**, *10*, 736-742. (b) Gupta, M. N. *Eur. J. Biochem.* **1992**, *205*, 25-32. (c) Wong, C.-W.; Whitesides, G. M. In *Enzyme Catalysis in Organic Solvents;* Tetrahedron Organic Chemistry Series Volume 12; Pergamon, Elsevier: Trowbridge, 1994; pp 22-25. (d) Gupta, M. N. In *Interface between Chemistry and Biochemistry*, Series: Exs 73; Jolles, P.; Jornwall, H. Eds., Birkhauser Verlag: Basel, Switzerland, 1995; pp 49-65.

(123) Rich, J. [illegible]; Dordick, J. S. *J. Am. Chem. Soc.* **1997**, *119*, 3245-3252.

(124) Review: (a) Waldmann, H.; Zelinsky, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 722-724. (b) Klibanov, A. M.; Xu, K. *J. Am. Chem. Soc.* **1996**, *118*, 9815-9819. (c) Wang, Y.-F.; Yakovlevsky, K.; Zhang, B. *J. Org. Chem.* **1997**, *62*, 3488-3495.

(125) Zaks, A.; Klibanov. A. M. *J. Biol. Chem.* **1988**, *263*, 3194-3201.

(126) (a) Broos, J.; Engbersen, J. F. J.; Sakodinskaya, I. K.; Verboom, W.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2899-2905. (b) Zheng, Y.-J.; Ornstein, R. L. *Protein Engineering* **1996**, *9*, 485-492. (c) Kim, J.; Kim, B. G. *Biotech. Bioeng.* **1996**, *50*, 687-692. (d) Noritomi, H.; Almarsson, Ö.; Barletta, G. L.; Klibanov, A. M. *Biotech. Bioeng.* **1996**, *51*, 95-99. (e) Dong, A.; Meyer, J. D.; Kendrick, B. S.; Manning, M. C.; Carpenter, J. F. *Arch. Biochem. Biophys.* **1996**, *334*, 406-414.

(127) Waygood, E. R. *Methods in Enzymology* Vol II, Colowick, S. P.; Kaplan, N. O. Eds.; Academic Press Inc.: New York, 1955; pp 836-846.

(128) (a) Vallee, B. L.; Auld, D. S. *Biochemistry*, **1990**, *29*, 5647-5659. (b) Coleman, J. E. *Annu. Rev. Biochem.* **1992**, *61*, 897-946. (c) Sigal, G. B.; Whitesides, G. M. *Bioorg. Med. Chem.* **1996**, *6*, 559-564. (d) Sumulan, S. L.; Casanova, J.; Alzyet, G.; Borràs, J.; Castiñeiras, A.; Supuran, C. T. *J. Inorg. Chem.* **1996**, *62*, 31-39.

(129) Grunwald, J.; Wirz, B.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 6732-6734.

(130) Bonnichsen, R. K.; Brink, N. G. *Methods in Enzymology,* Vol I; Colowick, S. P.; Kaplan, N. O. Eds.; Academic Press Inc.: New York, 1955; pp 495-503.

(131) Ryde, U. *Eur. Biophys. J.* **1996**, *24*, 213-221.

(132) (a) Ekland, H.; Samama, J.-P.; Wallèn, L.; Brüdén, C.-I. *J. Mol. Biol.* **1981**, *146*, 561-587. (b) Al-Karadaghi, S.; Cedergren, E.; Zeppezauer, E. S.; Petrantos, K.; Hovmöller, S.; Terry, H.; Dauter, Z.; Wilson, K. S. *Acta Crystallogr.* **1994**, *D50*, 793-807. (c) Al-Karadaghi, S.; Cedergren, E.; Zeppezauer, E. S.; Petrantos, K.;

Hovmöller, S.; Terry, H.; Dauter, Z.; Wilson, K. S. *Acta Crystallogr.* **1995**, *D51*, 805-813.

(133) Jones, J. B.; Beck, J. F. In *Applications of Biochemical Systems in Organic Chemistry, Vol 10, Techniques of Chemistry*; John Wiley ans Sons Inc.: New York, 1976; pp 236-376.

(134) Elsacker, P. C. V.; Lemière, G. L.; Lepoivre, J. A.; Alderweireldt, F. C. *Bioorg. Chem.* **1989**, *17*, 28-35.

(135) Itoh, S.; Terasaka, T.; Matsumiya, M.; Komatsu, M.; Oshiro, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3253-3254.

(136) Reviews: (a) Swern, D.; Mancuso, A. J. *Synthesis* **1981**, 165-185. (b) Tidwell, T. T. *Org. React.* **1990**, *39*, 297-572.

(137) Yoon, M. N.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem*, **1973**, *38*, 2786-2792.

(138) Hearing, Jr., V. J. *Methods in Enzymology*, Vol 142; Kaufman, P. Ed.; Academic Press Inc.: New York, 1955; pp 154-170.

(139) Espin, J.-C.; García-Ruiz, P. A.; Tuleda, J.; García-Cánovas, F. *Biochem. J.* **1998**, *331*, 547-551.

(140) Burton, A. G. *Biotech. Bioeng.* **1993**, *42*, 938-944.

(141) Broos, J.; Arends, R.; van Dijk, G. B.; Verboom, W.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1415-1417.

(142) Yates, P; Macas, T. S. *Can. J. Chem.* **1987**, *66*, 1-10.

(143) Richey Jr., H. G.; Domalski, M. S. *J. Org. Chem.* **1981**, *46*, 3780-3783.

(144) Krol, E. S.; Bolton, J. L. *Chemico-Biological Interactions*, **1997**, *104*, 11-27.

(145) Moreau, C.; Rouessac, F. *Bull. Soc. Chim. Fr.* **1973**, *12*, 3433-3436.

(146) Review: Olah, G. A. In *Friedel-Crafts and Related Reactions*; John Wiley and Sons: New York, 1963-1965.

(147) Review: Shine, H. J. In *Aromatic Rearrangements*; Elsevier: New York, 1967; pp 72-82, 365-368.

(148) Review: Fieser, L. F.; Fieser, M. In *Reagents for Organic Synthesis*, Volume 1; John Wiley and Sons: New York, 1967; pp 1287-1289.

(149) Review: Belen'kii, L. I. In *Chemistry of Organosulfur Compounds*; Ellis Horwood: Chichester, UK 1990; pp 193-228.

(150) Review: Eicher, T. *Supplement A: The Chemistry of Double-bonbed Functional Groups*, Volume 2, Pt. 2; John Wiley and Sons Inc.: New York, 1989; pp 621-693.

(151) Harris, J. M.; Bolessa, E. A.; Mendonca, A. J.; Feng, S.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1945-1950.

(152) Wildes, J. W.; Martin, N. H.; Pitt, C. G.; Wall, M. E. *J. Org. Chem.* **1971**, *36*, 721-723.

(153) Review: Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229-235.

(154) Balogh, V.; Fétizon, M.; Golfier, M. *J. Org. Chem.* **1971**, *36*, 1339-1341.

(155) Review: Barton, D. H. R.; Brewster, A. G.; Ley, S. V.; Rosenfeld, M. N. *J. Chem. Soc., Chem. Commun.* **1976**, 985-986.

(156) (a) Finley, K. T.; Colin, W. G.; Jones, D. W. In *Chemistry of Quinonoid Compounds*, Volume 1, Pt. 2 and Volume 2, Pt. 1; Patai, S.; Rappoport, Z. Eds.; John Wiley and Sons: Chichester, UK, 1988. (b) Liao, C.-C.; Chu, C.-S.; Lee, T.-H.; Rao, P. D.; Ko, S.; Song, L.-D.; Shiao, H.-C. *J. Org. Chem.* **1999**, *64*, 4102-4110. (c) Chu, C.-S.; Lee, T.-H.; Rao, P. D.; Song, L. D.; Liao, C.-C. *J. Org. Chem.* **1999**, *64*, 4111-4118.

(157) (a) Harley-Mason, J.; Laird, A. H. *J. Chem. Soc.* **1958**, 1718-1719. (b) Horner, L.; Dürkheimer, W. *Chem. Ber.* **1958**, *91*, 2352-2354.

(158) Ansell, M. F.; Gosden, A. F.; Leslie, V. J.; Murray, R. A. *J. Chem. Soc. (C)* **1971**, 1401-1414.

(159) Pappo, R.; Allen Jr., D. S.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478-479.

(160) Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863-927.

(161) Review: Sonnet, P. E. *Tetrahedron* **1980**, *36*, 557-604.

(162) (a) Levine, S. G. *J. Am. Chem. Soc.* **1958**, *80*, 6150-6151. (b) Larock, R. C. In *Comprehensive Organic Transformations*; VCH: New York; pp 715-716, 726.

(163) (a) Gilbert, J. C.; Weerasooriya, U. *J. Org. Chem.* **1982**, *47*, 4997-4998. (b) Gilbert, J. C.; Weerasooriya, U. *J. Org. Chem.* **1982**, *47*, 1837-1845.

(164) Review: Schlosser, M.; Christmann, F. K. *Synthesis* **1971**, 9-31.

(165) (a) Knowles, W. S.; Thompson, Q. E. *J. Org. Chem.* **1960**, *25*, 1031-1033. (b) Veysoglu, T.; Mitscher, L. A.; Swayze, J. K. *Stuttgart-New York* **1980**, 807-810.

(166) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd Ed.; Pergamon: New York; 1980.

(167) Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(168) Li, Z. *Biosynthetic Studies on the Polyketides, Fungichromin and Dehydrocurvularin: Incorporation of Advanced Precursors*, Ph. D. Thesis, University of Alberta, 1992.

(169) Although the compound is a single stereoisomer and crystallizes in a chiral space group, the X-ray results can only be used to determine the relative stereochemistry. The absolute stereochemistry is deduced from the known absolute stereochemistry of lovastatin (**3**) and compactin (**4**), which are biosynthetic derivatives.

(170) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467-473.

(171) Sheldrick, G. M. *SHELXL-93*. Program for crystal structure determination. University of Göttingen, Germany, 1993. Refinement on F_o^2 for all reflections (all of these having $F_o^2 \geq -3\sigma(F_o^2)$). Weighted *R*-factors wR_2 and all goodnesses of fit *S* are based on F_o^2; conventional *R*-factors R_1 are based on F_o, with F_o set to zero for

negative F_o^2. The observed criterion of $F_o^2 > 2\sigma(F_o^2)$ is used only for calculating R_1, and is not relevant to the choice of reflections for refinement. *R*-factors based on F_o^2 are statistically about twice as large as those based on F_o, and *R*-factors based on ALL data will be even larger.

(172) Block, M. H.; Cane, D. E. *J. Org. Chem.* **1998**, *53*, 4923-4928.

(173) (a) Simon, E. J.; Shemin, D. *J. Am. Chem. Soc.* **1953**, *75*, 2520. (b) von Stackelgerb, M. *Angew. Chem.* **1953**, *65*, 186-187. (c) Wilson, I. B. *J. Am. Chem. Soc.* **1952**, *74*, 3205.

(174) (a) Fenton, H. J. H. *J. Chem. Soc., Trans.* **1905**, *87*, 804-818. (b) Fenton, H. J. H. *J. Chem. Soc., Trans.* **1894**, *65*, 899-910.

(175) Fenton, H. J. H. *Biochem. Prep.* **1955**, *4*, 56-59.

(176) House, H. O.; Jones, V. K.; Frank, G. A. *J. Org. Chem.* **1964**, *29*, 3327-3333.

(177) Emmons, W. D.; Lucas, G. B. *J. Am. Chem. Soc.* **1955**, *77*, 2287-2288.

(178) Crimmins, M. Y.; Jung, D. K.; Gray, J. L. *J. Am. Chem. Soc.* **1993**, *115*, 3146-3155.

(179) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4743-4763.

(180) Isler, O.; Gutmann, H.; Montavon, M.; Rüegg, R.; Ryser, G.; Zeller, P.; *Helv. Chim. Acta* **1957**, *40*, 1242-1249.

(181) (a) Do Amaral, A. T.; El Seoud, O. A.; Do Amaral, L. *J. Org. Chem.* **1975**, *40*, 2534-2536. (b) Curran, D. P.; Luo, Z.; Degenkolb, P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2403-2408.

(182) (a) Mitoma, C.; Saito, T.; Howd, R. A.; *Xenobiotica* **1977**, *7*, 165-179. (b) Xu, Y.; Liu, S.; Ma, B.; Wang, S. *Zhongguo Yaowu Huaxue Zazhi* **1997**, *7*, 240-245.

(183) (a) Baddar, F. G.; Abdel, W. S.; Awad, B. M.; Guindy, N. M.; Wahba, M.; Abdel, M. B. A. *J. Chem. Soc. B* **1970**, *4*, 739-745. (b) Li, T.-S.; Li, A.-X. *J. Chem. Soc., Perkin Trans. 1* **1998**, *1*, 1913-1918.

(184) (a) Wenkert, E.; Alonso, M. E.; Gottlieb, H. E.; Sanchez, E. L.; Pellicciari, R.; Cogolli, P. *J. Org. Chem.* **1977**, *42*, 3945-3949. (b) Curzu, M. M.; Pinna, G. A.; Cignarella, G.; Barlocco, D.; Demontis, M. P. *Collect. Czech. Chem. Commun.* **1991**, *56*, 2494-2499.

(185) Inoki, S.; Mukaiyama, T. *Chem. Lett.* **1990**, *1*, 67-70.

(186) Mihel, I.; Orlovic, M.; Polla, E.; Borcic, S. *J. Org. Chem.* **1979**, *44*, 4086-4090.

(187) Hasegawa, E.; Curran, D. P. *Tetrahedron Lett.* **1993**, *34*, 1717-1720.

(188) Larock, R. C.; Leung, W. Y.; Stolz-Dunn, S. *Tetrahedron Lett.* **1989**, *30*, 6629-6632.

(189) Buergi, C.; Ruedi, P. *Helv. Chim. Acta* **1993**, *76*, 1901-1915.

(190) (a) Cardona, M. L.; Fernandez, M. I.; Garcia, M. B.; Pedro, J. R. *Tetrahedron*, **1986**, *42*, 2725-2730. (b) Takaoka, E.; Yoshikawa, N.; Yamada, Y. M. A.; Sasai, H.; Shibasaki, M. *Heterocycles* **1997**, *46*, 157-163.